KERATOCONJUNCTIVITIS SICCA IN RHEUMATOID ARTHRITIS

by

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M.D. Thesis
University of Edinburgh
1970

I dedicate this thesis to my wife whose encouragement has sustained me throughout its completion.

" My object has always been to utilise my time in gratutious work if I was otherwise unoccupied. So the next move in this direction was to accept the position of Assistant Surgeon to the Glasgow Ophthalmic Institution ....."

From "Reminiscences of an old physician" by Robert Bell, M.D., F.R.F.P.S. - (John Murray, Albemarle Street West, London, 1924).

PREFACE

My interest in the ocular manifestations of rheumatic disease was aroused just over five years ago, when I was consulted at the then newly opened Centre for Rheumatic Diseases, Glasgow, regarding the eye complications of a patient with rheumatoid arthritis. Mrs. A. McC. was a 54-year old housewife who had had rheumatoid arthritis for 20 years and had been bed-ridden for 4 years despite intensive systemic therapy including corticosteroids. She gave a three year history of ocular discomfort, extreme photophobia and recurrent tenacious discharge from both eyes unrelieved by topical therapy. I discovered that she suffered from severe keratoconjunctivitis sicca and in addition had developed a central corneal perforation in one eye.

The magnitude of this patient's eye disease stimulated me to study the problems which form the basis of this thesis.

Some of the data contained in the thesis has been published or communicated to learned societies.

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SUMMARY

In his book "The Struggle for Survival", Lord Moran recorded of Sir Winston Churchill on the occasion of his 80th birthday "A fine disregard for common sense has marked his earthly pilgrimage". I have often thought that the same might be true of the most protean of ophthalmological diseases, Sjøgren's syndrome.

Sjøgren's syndrome is a chronic, benign, idiopathic disease comprising the triad of keratoconjunctivitis sicca (K.C.S.), xerostomia, and rheumatoid arthritis (R.A.) or other connective tissue disorders. My thesis presents new facts about this enigmatic condition, and contains chapters on diagnosis, histopathology, prevalence in rheumatoid and non-rheumatoid populations, relationship to clinical and laboratory features of rheumatoid arthritis, and clinical and immunological associations with other autoimmune diseases, such as Hashimoto's thyroiditis, pernicious anaemia, and primary idiopathic Addison's disease of the adrenal gland. In addition, chapters are included dealing with the therapy of K.C.S. with special reference to the effects of treatment on the bacterial, fungal and viral flora of the conjunctival sac.

Chapter I gives a historical outline of Sjøgren's syndrome, and in Chapter II I describe a computer-assisted mathematical model for diagnosing K.C.S. based

on an analysis of symptoms and signs. This clinical diagnostic index will perhaps be of value to clinicians who have no specialised ophthalmological expertise in epidemiological research. In Chapter II, I also present a critical appraisal of diagnostic tests, such as Schirmer's tear test and biomicroscopy, for K.C.S.

Despite the fact that Sjøgren's syndrome has been studied for nearly half a century, I discovered on perusal of the literature a remarkable paucity of information on the histopathology of the lacrimal glands. Chapter III describes a detailed pathological study on lacrimal gland biopsies in patients with definite K.C.S., patients with possible K.C.S., and in patients with no evidence of ocular disease. This study has shown that lacrimal gland histology is identical in patients with Sjøgren's syndrome uncomplicated by R.A. or other connective tissue disease (sicca syndrome) and in patients with Sjøgren's syndrome complicated by R.A. Severity and extent of lacrimal gland inflammation has been found to correlate with the duration of ocular symptoms, but long-term corticosteroid therapy had no apparent effect on the histological appearances.

Since autoimmunity is common in the elderly and since the aging process itself has been attributed to an autoimmune mechanism, I thought it of interest to study the prevalence of K.C.S. in elderly subjects, and this work is presented in Chapter IV. The diagnosis

of K.C.S. was made in a surprisingly high number of the elderly subjects examined, and the absence of any relationship with autoantibodies which are commonly found in younger persons with K.C.S. with or without R.A., has led me to the conclusion that in the elderly K.C.S. is probably a result of acinar atrophy rather than chronic inflammation.

In order to try to delineate possible pathogenetic factors in producing K.C.S., I undertook a detailed and comprehensive study of K.C.S. in 893 patients with R.A., relating the presence of K.C.S. with the clinical and laboratory features of the rheumatoid disease. This study involved mathematical analysis, including discriminant analysis, and the results which are presented in Chapter V show that the development of K.C.S. is related to the severity and duration of the arthritis and to the extent of non-articular rheumatoid complications.

Because of the high prevalence of autoantibodies in Sjøgren's syndrome and because of the histological similarities between the lacrimal and salivary glands in this disease and the thyroid gland in Hashimoto's thyroiditis, gastric mucosa in pernicious anaemia, and adrenal gland in primary idiopathic Addison's disease, I decided to investigate the possible associations between K.C.S. and these organ-specific autoimmune diseases. The results are discussed in Chapter VII.

Studies of the relationship between salivary duct autoantibody and K.C.S. and histological changes in

the labial mucosa are described in Chapter VI. The findings indicate that salivary duct antibody is not peculiar to Sjøgren's syndrome, but appears to be related to the rheumatoid process. Tests for this antibody consequently have no diagnostic value for the ophthalmologist.

Although it has frequently been stated in the literature that conjunctival infection is common in K.C.S., a careful review of all available literature failed to identify one publication where the prevalence of this complication had been ascertained. I, therefore, decided that a careful documentation and investigation of ocular infection in K.C.S. would be worthwhile, and in Chapter VIII I describe investigations of bacterial, fungal and viral flora in K.C.S. The conclusion of this study is that bacterial and fungal infection is significantly high in untreated K.C.S., but that lubricant therapy greatly reduces this secondary infection. A controlled study of combined antibiotic-corticosteroid topical therapy revealed a high fungal infection complication rate. In Chapter IX I conclude the thesis by reviewing my personal experience of various forms of treatment of K.C.S.

ACKNOWLEDGEMENTS

A work of this nature requires the goodwill, co-operation and encouragement of colleagues in many branches of clinical and laboratory medicine. In the interests of brevity, I would like to mention but a few.

Professor Wallace S. Foulds, University Department of Ophthalmology, Tennent Institute, for the use of clinical and technical facilities

Dr. Robert Leishman, Senior Consultant Ophthalmologist, Southern General Hospital, for reading some of the drafts and for helpful criticism

Professor Robert Goudie, University Department of Pathology, Glasgow Royal Infirmary, for education and guidance in the application of autoimmune phenomena

Professor E.M. McGirr, University Department of Medicine, Glasgow Royal Infirmary, for access to patients with thyroid diseases

Professor A.S. Douglas, University Department of Medicine, Glasgow Royal Infirmary, for access to patients with pernicious anaemia

Dr. W. Watson Buchanan, University Department of Medicine, Glasgow Royal Infirmary, Centre for Rheumatic Diseases, Baird Street, for allowing me to examine patients with rheumatoid arthritis and related diseases and for constant encouragement.

On no account should I fail to record the help of Mr. Louden Brown, Medical Illustration Department, Southern General Hospital, and Miss Dorothy Ritchie,

Centre for Rheumatic Diseases, for preparing the illustrations, and Mrs. Jean Sharp, Centre for Rheumatic Diseases, for her patience in typing this thesis.

Finally, my sincere thanks to all the hundreds of patients who permitted me to examine them, sometimes frequently. I hope that my efforts will benefit them in some small measure.

CONTENTS

CHAPTER I

HISTORICAL SECTION

SJØGREN'S SYNDROME is a chronic benign disease of unknown aetiology, which consists of the triad of keratoconjunctivitis sicca, xerostomia and rheumatoid arthritis or another connective tissue disease. The keratoconjunctivitis sicca and xerostomia of the syndrome are caused by progressive atrophy of the acinar parenchyma with round cell infiltration of the lacrimal and salivary glands. Thus, specific infiltrations of these glands such as may occur in sarcoidosis, tuberculosis, lymphomas and Waldenstrom's macroglobulinaemia are excluded by definition. It is generally accepted that only two of the three components are necessary for the diagnosis. When keratoconjunctivitis sicca and xerostomia are present alone, the term sicca syndrome is often used.

An ocular disorder in which small filaments were found attached to the cornea and suspended over its surface was described in 1882 by Leber. Many causes were suspected for this entity which was named filamentary keratitis. It was nearly 50 years later before attention was focused on the combination of filamentary keratitis and reduced tear secretion (Stock, 1924) although both disorders had been recorded separately. Thus in 1888 Hadden gave an account of a 63-year old female patient who could not produce tears even after inhaling "strong ammonia". However, he detected no corneal or conjunctival abnormality and clinical examination of the lacrimal and salivary glands was normal. The main burden of his report was the description of extreme dryness of his patient's tongue, cheeks, palate, pharynx and nasal mucosa.

The first description of an arthritic patient who had filamentary keratitis appears to have been made by Fischer in 1889. However, the possible significance of a relationship between arthritis and filamentary keratitis was not emphasized and was not reported again for nearly half a century when Houwer in 1927 described 10 patients with this ocular idsorder, six of whom had chronic arthritis.

It appears from my perusal of the literature, therefore, that before the year 1900, nearly all of the components of what was to become known as Sjøgren's syndrome (1933) were on record although the disease had not been defined.

The complete picture of keratoconjunctivitis sicca, xerostomia and chronic arthritis was unveiled gradually between 1919 and 1933. Fuchs in 1919 described a 54-year old female patient who complained of dry eyes and a dry mouth and who developed unilateral parotid gland swelling. Changes in the cornea were not reported but he noted that a thick secretion, which he believed to be from the Meibomian glands, covered the conjunctivae in both eyes. Deutchsman described a similar case in 1921 and in 1926, Gougerot reported three patients who were suffering from progressive atrophy of the "mucous" glands of the conjunctivae and mouth, of the salivary glands and in some degree the naso-pharyngeal, laryngeal and vulvar glands. Stock (1924) attributed filamentary keratitis in two of his female patients to diminished tear secretion and Schöninger later that year described the same two patients in full. One of them had been unable to cry since birth, the other since aged 18 years. Regardless of the stimulus, tear secretion was minimal in these two patients. Their conjunctival sacs contained long yellowish-white threads which could be separated from the conjunctival and corneal surfaces only with difficulty. The cornea of one eye in either patient was dull and the visual acuity reduced. Both patients complained intermittently of foreign body sensation and burning feelings in their eyes and photophobia was extreme. Important contributions from Houwer followed in 1927, 1928 and 1929. He described 10 patients

suffering from filamentary keratitis, eight female and two male, nearly all of whom were past middle-age. More than half of them had chronic arthritis, the exact form of which was not defined. Spontaneous remissions and variations in ocular pathology in the same patient at different times were emphasized. Furthermore, Houwer pointed out that the conjunctiva was almost always involved and appeared thickened and hyperaemic. The corneal filaments, usually concentrated over the lower third of the cornea, were often tiny and at times absent. Small superficial corneal defects with or without infiltration of the underlying stroma were present even during remissions. The probability is that Houwer included some early and mild cases of "keratoconjunctivitis sicca" (Sjøgren, 1933) in his series casting his net wider than previous investigators. Confirmation of this superficial punctate keratitis came from Betsch in 1928. Two female patients aged 46 and 53 years developed reduced tear secretion and intermittent filamentary keratitis. Betsch reiterated the view that filaments were characteristic of relapses, superficial punctate defects were present constantly. In addition, one of his patients had bilateral parotid gland swelling, hoarseness and severe dryness of the mouth and throat. Neither patient had arthritis. However, Isakowitz in 1928 described a female patient suffering from a peri-arthritis who developed reduced tear secretion and filamentary keratitis; and in 1932 Wissmann reported four of six patients who had

filamentary keratitis and arthritic changes.

In 1933 Henrik Sjøgren published his now famous monograph "Zur Kenntnis der Keratoconjunctivitis Sicca" which was translated into English by J. Bruce Hamilton (1943) as "A New Conception of Keratoconjunctivitis Sicca". Sjøgren described in detail a series of 19 women only two of whom were under 40 years of age. All had reduced tear secretion and 13 had arthritis. Sjøgren popularised the use of the vital staining dye bengal rose and this enabled him to demonstrate the constant and extensive involvement of the conjunctiva. "It has been revealed that the eye is hereby stained in a particularly characteristic manner. The part of the eye corresponding to the rima region stains an intense red. If the eyelids are held apart, a bright red, sharply defined triangle with the base towards the limbus is noticed at each side of the cornea. On the cornea the filaments and epithelial scales are stained; furthermore, the cornea takes up colour to a considerable extent in regions which, without staining, look normal. It emerges from this that not only the cornea but also the conjunctiva is extensively diseased". With this in mind, Sjøgren proposed the more comprehensive term - keratoconjunctivitis sicca. He noted also the diminution in salivary secretion and the frequency of general symptoms and concluded that "we have to deal here with a general disease". Sjøgren reasserted this belief in subsequent papers (1935 a,b,c, 1936, 1937, 1938 and 1948) and as early as 1935 (c)

stated that the general disease was in most instances a chronic rheumatic polyarthritis.

By 1951, Sjøgren had diagnosed 80 cases of keratoconjunctivitis sicca, 50 of whom (62 per cent) had arthritis. Most of these 50 patients had in fact rheumatoid arthritis. The frequency of rheumatoid arthritis in other series varies from 17 per cent of 121 patients (Henderson, 1950) to 87 per cent of 23 patients (Godtfredson, 1947). This variation is due partly to selection of cases and partly to the intensity of the search for evidence of arthritis. Henderson (1950) admitted that not all of his patients had x-ray examinations and that the frequency of arthritis reported in his series might be unduly low. Bunim (1961) reported 40 cases of keratoconjunctivitis sicca in which 17 had definite rheumatoid arthritis (42.5 per cent) and a further two had probable rheumatoid arthritis. Bloch, Buchanan, Wohl and Bunim (1965) in a comprehensive study of 62 patients suffering from keratoconjunctivitis sicca discovered definite rheumatoid arthritis in 30 patients (50 per cent) and probable rheumatoid arthritis in another two patients. Conversely, the frequency of keratoconjunctivitis sicca in patients suffering from rheumatoid arthritis has been examined by several investigators. Stenstam (1947) was the first to report a sizeable group of 495 patients of whom 9.5 per cent had dry eyes. Holm (1949) discovered that 14.2 per cent of 500 arthritic patients examined after instillation of rose bengal stain had keratoconjunctivitis

sicca. However, the types of arthritis in Holm's series were not defined. Nevertheless, his incidence of dry eyes tallies closely with that in Thomson and Eadie's group of 1956 in which 14.3 per cent of 210 patients suffering from rheumatoid arthritis had keratoconjunctivitis sicca. According to some reports, a higher incidence may be encountered in patients with advanced joint disease (Reader, Whyte and Elines, 1951; Lackington, Charlin and Gormay, 1951). However, when the full range of rheumatoid arthritic patients is examined the incidence remains between 9 and 14 per cent (Gaulhofer, 1954).

Rheumatoid arthritis is the largest member of the confederacy of disorders commonly termed, the connective tissue diseases. These include systemic lupus erythematosus, progressive systemic sclerosis (diffuse scleroderma), polyarteritis nodosa and dermatomyositis (polymyositis). The concept that a number of clinical conditions might be grouped together on grounds of common pathology was advanced by Klemperer, Pollack and Baehr in 1942. They demonstrated fibrinoid change starting in the collagen fibres of the connective tissues in some cases of systemic lupus erythematosus and diffuse scleroderma. The lesions extended to involve the cells and ground substance as well as the collagen fibres and the use of the term connective tissue disease eventually became universal.

In 1872, Kaposi described a patient with a dry fissured tongue and cheeks, parotitis and systemic lupus

erythematosus. Sixteen years later Hutchison (1888) reported a case of probable systemic lupus erythematosus associated with ocular symptoms very suggestive of keratoconjunctivitis sicca. Yet again many years were to elapse before interest was revived in a possible connection between Sjøgren's syndrome and connective tissue diseases other than rheumatoid arthritis.

Single instances of keratoconjunctivitis sicca and systemic lupus erythematosus occurring in the same patient were reported in the 1950's (Morgan, 1954; Schapnosnik, Bergna and Conti, 1955; Ramage and Kinnear, 1956; Bain, 1960). MacLean and Robinson (1954) found lupus erythematosus cells in the blood of a patient suffering from rheumatoid arthritis and keratoconjunctivitis sicca and Heaton (1959) described similar cells in 28 consecutive patients suffering from Sjøgren's syndrome. Nevertheless, comprehensive examinations of large series of patients suffering from systemic lupus erythematosus, even when full ocular and oral examinations appear to have been carried out, have failed to reveal the components of Sjøgren's syndrome in the majority of cases (Hill, 1957). In addition, the inclusion of systemic lupus patients in series of Sjøgren's disease has occurred very rarely (Stoltze, Hanlen, Pease and Henderson, 1960). However, non-suppurative parotitis was reported by Shearn and Pirofsky (1952) in four of 34 patients and in two of 138 patients examined by Harvey, Schulman, Tumulty, Conley and Schoenrich (1954). Later Shearn (1960)

extended his observations to include 6 of 83 patients suffering from systemic lupus (7.2 per cent) and microscopic examination of biopsy material from the parotid gland of one of his patients with parotitis disclosed a pattern compatible with early Sjøgren's disease.

Reports of single cases of Sjøgren's syndrome occurring in patients suffering from progressive systemic sclerosis also began to appear after the Second World War (Leriche, 1947; Harrington and Dewar, 1951; Caughey and Richardson, 1952; Ramage and Kinnear, 1956, and Piazzesi, 1956). Two such patients were described by Shearn (1960) in one of whom autopsy findings confirmed the diagnosis. Authorities dealing with large series of progressive systemic sclerosis failed to mention Sjøgren's syndrome (Leinwand, Duryce and Richter, 1952; Beigelman, Goldner and Baylis, 1953) until Stava (1958) noted keratoconjunctivitis sicca in five and xerostomia in two of 65 patients. Kirkham (1969) studied 9 patients suffering from progressive systemic sclerosis and was satisfied with the diagnosis of keratoconjunctivitis sicca in one patient. However, two others had evidence of reduced tear flow and conjunctival staining with rose bengal dye. Conversely, patients with progressive systemic sclerosis have been included in large Sjøgren series (Stoltze and others, 1960; Bloch, Buchanan, Wohl and Bunim, 1965).

Typical polyarteritis nodosa and severe keratoconjunctivitis sicca was diagnosed in a patient

by Ramage and Kinnear (1956) and both diseases were confirmed at post mortem examination.

As far as I can judge this is the only completely convincing record of the association of the two diseases. In 1954 Cardell and Gurling described a 49-year old female rheumatoid patient who had keratoconjunctivitis sicca. At autopsy polyarteritis nodosa, which had not been suspected, was diagnosed. This probably was the arteritis commonly found in rheumatoid arthritis (Cruikshank, 1954). Shearn (1961) described a 36-year old female patient who in addition to keratoconjunctivitis sicca and xerostomia developed enlarged lacrimal, parotid and cervical lymph nodes. Biopsy of a cervical lymph node from this patient showed changes consistent with "panarteritis". The evidence, therefore, in this patient described by Shearn of polyarthritis nodosa is tenuous. Two patients with polyarteritis nodosa are included in the 139 Sjøgren patients described by Stoltze, et al (1960) but no details are given.

Bloch and his colleagues (1965) reported that four of their 62 patients with Sjøgren's syndrome had polymyositis. The patients were carefully examined for evidence of rheumatoid arthritis and only one of them might have had it in the past. The only other report of the association of Sjøgren's syndrome and polymyositis concerns a 45-year old female patient who had polymyositis and rheumatoid arthritis and who developed lacrimal and parotid adenitis (Fox, 1966).

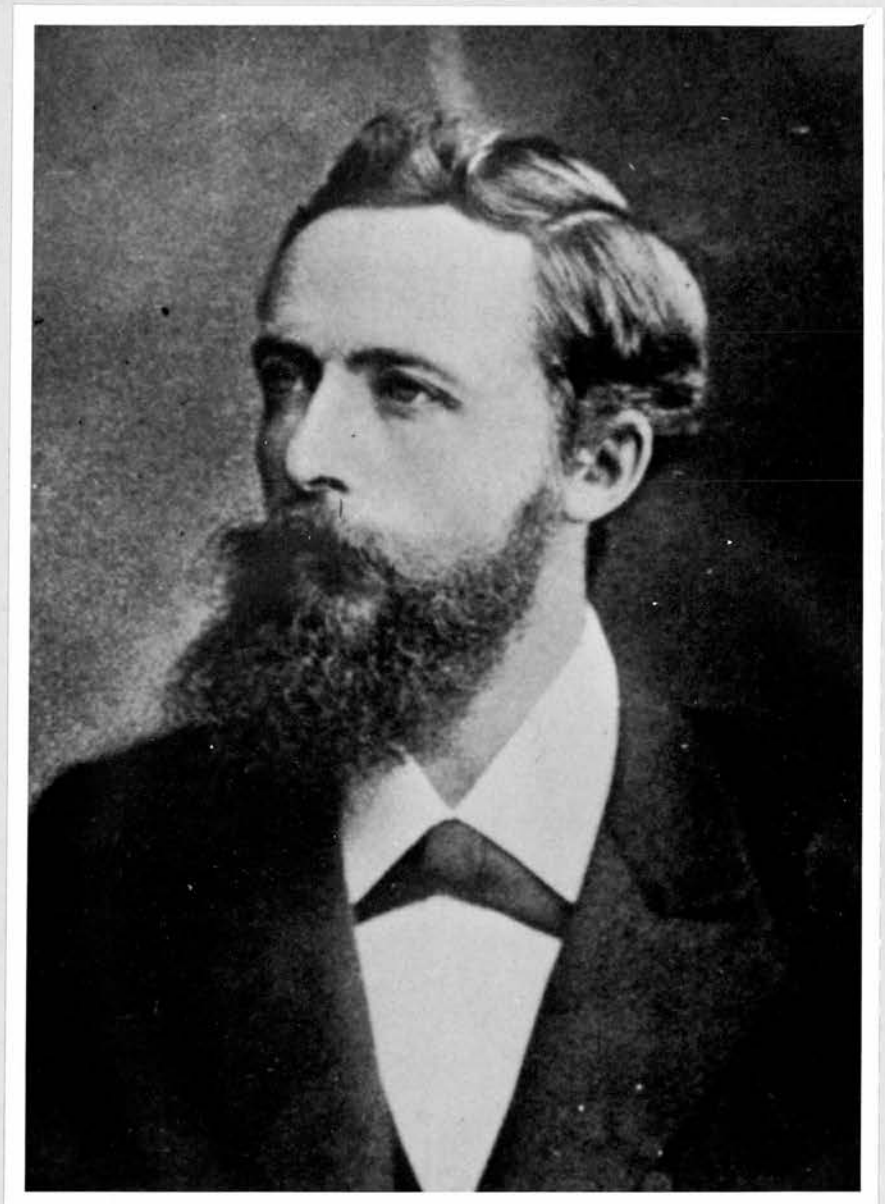

Fig.1,1 von Mikulicz

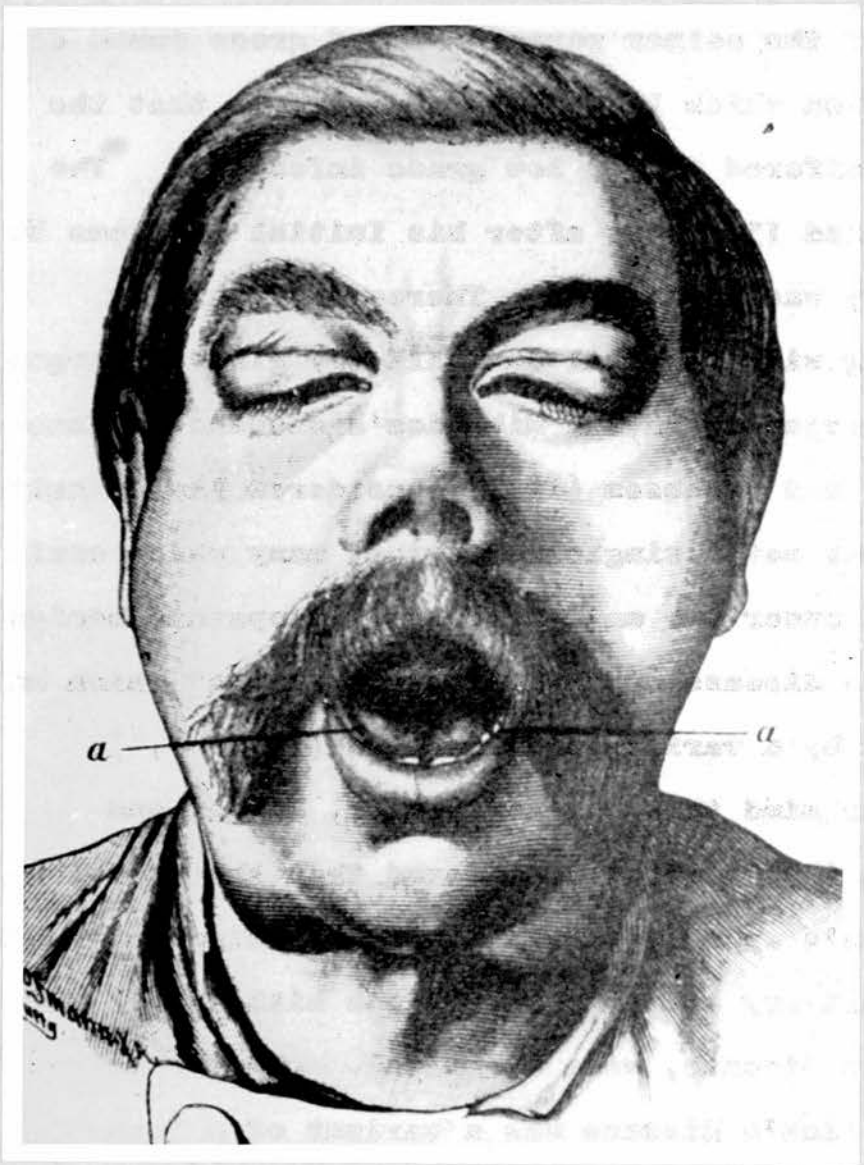

Fig.1,2 von Mikulicz's patient.

Lacrimal and salivary gland enlargement.

(a) indicates sublingual glands.

In 1888, von Mikulicz (Fig.1,1) recorded the case history of a 42-year old Prussian farmer who developed enlargement of the lacrimal and salivary glands without any evidence of diminished secretion (Fig.1,2). Biopsy of the lacrimal and salivary glands revealed atrophy of the aciner parenchyma and gross round cell infiltration which led Mikulicz to deduce that the patient suffered from a low grade infection. The patient died 13 months after his initial symptoms but no autopsy was performed. Thereafter patients presenting with lacrimal and salivary gland enlargement were diagnosed as having Mikulicz's disease. However, Schaeffer and Jacobsen (1927) considered that Mikulicz's disease was not a single entity but many which could be described under two main headings, idiopathic benign Mikulicz's disease or "Mikulicz's syndrome" which may be caused by a variety of disorders (Table 1,1). Bearing in mind this classification, Morgan and Castlemen (1953, 1954) considered that the histology in Sjøgren's syndrome and in chronic benign enlargement of the salivary and lacrimal glands hitherto called Mikulicz's disease, were identical. They concluded that Mikulicz's disease was a "variant of a larger symptom complex, Sjøgren's syndrome".

The confusion in the literature concerning the relationship of Mikulicz's disease and Sjøgren's syndrome has a close parallel with Reidel's thyroiditis and Hashimoto's thyroiditis. Reidel described a "hard" goitre in a patient and the brevity of the histological

RELATIONSHIP OF MIKULICZ'S DISEASE AND SJOGREN'S SYNDROME

MIKULICZ (1888)

42 year old farmer with bilateral enlargement of lacrimal and salivary glands, but no impairment of secretion. Biopsies of lacrimal and submaxillary glands showed massive round-cell infiltration and atrophy of acinar parenchyma.

SCHAFFER AND JACOBSEN (1927)

Classification :

1. Mikulicz's disease proper.
2. Mikulicz's syndrome (Leukaemia, lymphosarcoma, tuberculosis, sarcoidosis, lead and iodine poisoning)

SJOGREN (1933)

Nineteen women with keratoconjunctivitis sicca, xerostomia and salivary gland enlargement. Thirteen had arthritis.

MORGAN AND CASTLEMAN (1953, 1954)

Concluded that Mikulicz's disease and Sjogren's syndrome were the same condition.

Table 1,1

report is uncannily like that of Mikulicz's report on the salivary glands of his patient. In neither instance was there sufficient clinical or pathological evidence to adequately define the disease. Hashimoto, like Sjøgren, on the other hand described his 4 cases of goitre with considerable attention to clinical and histological detail, and noted that failure of the thyroid gland may lead to hypothyroidism. As with the Mikulicz/Sjøgren controversy the Reidel/Hashimoto controversy continued for many years until finally settled by pathological studies.

SUMMARY

SJØGREN'S SYNDROME consists of the triad of keratoconjunctivitis sicca, xerostomia and rheumatoid arthritis though only two of these three components are necessary for the diagnosis to be made. There is growing evidence that other connective tissue diseases may on occasion replace rheumatoid arthritis in the syndrome. However, the incidence of these diseases in Sjøgren's syndrome is much lower than that of rheumatoid arthritis and the converse is also true. Systemic lupus erythematosus, progressive systemic sclerosis, polyarteritis nodosa and polymyositis have each been implicated in order of decreasing frequency.

There is now incontrovertible evidence that Sjøgren's syndrome and Mikulicz's disease are variants of the same pathological process.

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CHAPTER II

OPHTHALMOLOGICAL EXAMINATION

The dry eye of Sjøgren's syndrome is the result primarily of reduced secretion from the lacrimal and accessory lacrimal glands. Hypofunction of the tear forming glands may be measured by means of Schirmer tear tests. However, before a diagnosis of keratoconjunctivitis sicca can be made, the eye must be seen to have suffered as a result of reduced tear secretion as expressed in the form of specific staining patterns with the vital dye rose bengal (Sjøgren, 1933).

This chapter deals with factors influencing Schirmer's tear tests, the value of symptoms and signs tabulated by the physician, the range of biomicroscopic changes commensurate with a diagnosis of keratoconjunctivitis sicca and the possibilities of the lysozyme test.

PATIENTS STUDIED

Patients attending the Centre for Rheumatic Diseases, Glasgow, between June 1965 and January 1970 were examined by the author at eye clinics held specifically for this purpose at the Ophthalmic Institute, Glasgow (1965-1966) and the University Department of Ophthalmology, Western Infirmary, Glasgow (1966-1970). In addition, all of the patients admitted to the rheumatic hospital were screened under inpatient conditions and referred to the outpatient clinics as required. The diagnosis, clinical and laboratory details were extracted by the author and recorded on computer punch cards. The result of this exercise is the subject of Chapter V which deals with keratoconjunctivitis sicca in rheumatoid arthritis.

OPHTHALMOLOGICAL EXAMINATIONS

The first eye examination included recording of visual acuity, refractive errors, evaluation of ocular adnexa, pupil reactions and eye movements. The conjunctivae, corneae and anterior segment of the eye were examined without and with the aid of a slit lamp and the lens, vitreous and fundus were viewed through dilated pupils.

Schirmer Tear Tests

In 1903, Schirmer devised a simple test for measuring tear secretion. He utilised strips of filter paper 5 mm. wide by 15 mm. long which he folded at a right angle 5 mm. from the end to be inserted into the un-anaesthetized conjunctival sac, at the outer third of the lower lid. Both eyes were tested simultaneously and at the end of 5 minutes, the length of paper moistened by tears was measured. Patients normally moisten more than 15 mm.; less than 15 mm. wetting requires investigation. This test is referred to as the Schirmer I tear test or unforced test to distinguish it from the Schirmer II or forced tear test in which there is an added stimulus to tear secretion. To obviate the tedious task of cutting hundreds of filter papers to the correct size, I used standardised Schirmer Tear Test Kits as devised by Iso-Sol Company,

Lindenhurst, New York (Halberg and Berens, 1961).

The unforced test is a useful screening procedure for evidence of reduced tear secretion but as first noted by Schirmer in 1903 and later confirmed by others (Sjøgren, 1933; de Roetth, 1941; Henderson and Prough, 1950, Wright and Meger, 1962) less than 15 mm. of wetting may be recorded by this method in a proportion of normal subjects. In particular, caution has to be exercised in interpreting the results of Schirmer I tear tests in rheumatoid arthritic patients living in a warm dry atmostphere.

The Effect of Temperature and Humidity on the Schirmer I Tear Test

All of the inpatients at the Centre for Rheumatic Diseases in Glasgow have Schirmer I tear tests a few days after admission to the hospital. Early in 1966, I noted that the frequency of positive unforced tear tests was very high. Those patients with Schirmer I tests of less than 15 mm. wetting were re-examined one week later at the ophthalmic outpatient department. The majority on this second occasion gave normal readings; the remainder were shown later to be suffering from keratoconjunctivitis sicca. A third unforced Schirmer test was carried out the following week in the rheumatic hospital. Once again, the same patients demonstrated lower than 15 mm. wetting of the filter paper strips in 5 minutes when no added stimulus was used.

Material and Methods

At the time this phenomenon came under scrutiny, 338 inpatients comprising 250 females and 88 males had been examined (age range 18-72 years, mean age 56 years). Apart from those later shown to have keratoconjunctivitis sicca, 65 patients (20%) had positive Schirmer I tear tests. 123 eyes were suspect, 78 of them reading less than 5 mm. in 5 minutes (60%). The 65 patients were re-examined, one week later, in an

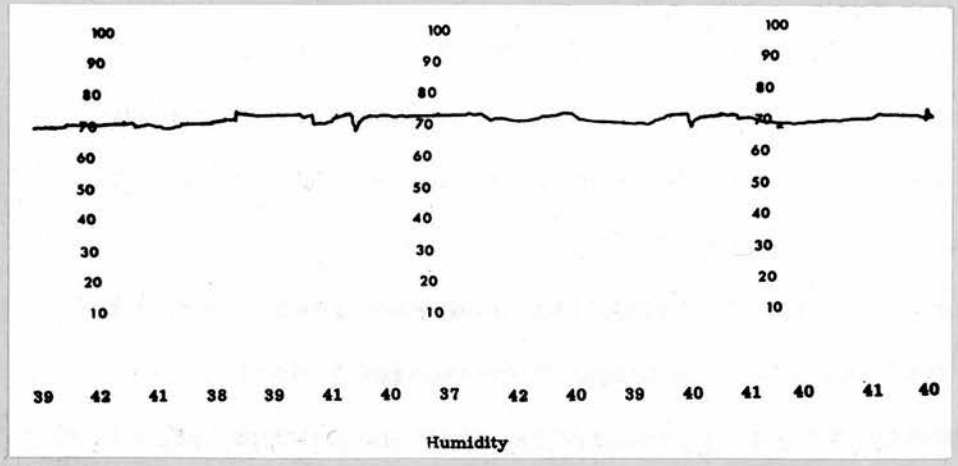


Fig.II,1 Temperature and humidity in rheumatic wards

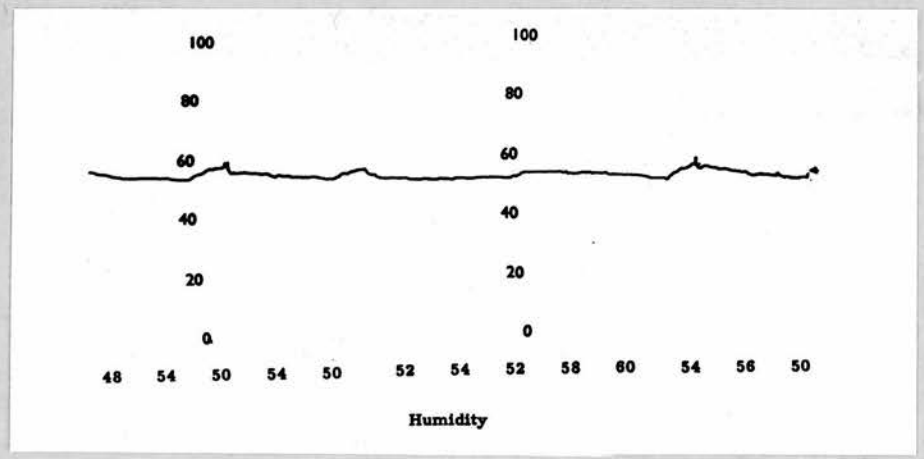


Fig.II,2 Temperature and humidity in eye outpatient department

ophthalmic outpatient department. In every case, the Schirmer I tear test was normal and there was no evidence of keratoconjunctivitis sicca. The suspect patients were returned to the rheumatic centre where another week elapsed before the third tear test was carried out. Again reduced unforced tear test readings were obtained, this time from 110 of the eyes, 75 of them reading less than 5 mm. in 5 minutes (68%).

Thermographic and humidity records (self recording barometer and whirling string hydrometer) were made simultaneously in both hospitals over a period of 20 days. Fig.II,1 shows part of the tracing from the rheumatic hospital, where the mean temperature was 72.3°F. (22.3°C.) and the humidity 40.5. Fig.II,2 shows part of the tracing from the ophthalmic outpatient department, where the mean temperature over the same period was 57.4°F. (14.1°C.) and the humidity 48. While these recordings were being made, 12 of the rheumatic centre patients who had demonstrated positive Schirmer I tear tests were given a further six unforced tear tests, three in the ophthalmic hospital, three in the rheumatic hospital.

Results

Six recordings of the unforced tear test were thus available for 12 patients, none of whom was suffering from keratoconjunctivitis sicca. The results show that

Case No.	Sex	Three Recordings (average of two eyes)	
		At Rheumatic Hospital	At Ophthalmic Hospital
1	F	11, 12, 11	15, 18, 18
2	F	8, 7, 7	20, 20, 19
3	M	4, 8, 7	16, 18, 17
4	F	3, 7, 5	20, 18, 20
5	F	8, 12, 11	20, 20, 20
6	M	12, 11, 12	22, C.W., C.W.
7	M	14, 15, 10	18, 18, 20
8	M	15, 11, 14	19, 20, 20
9	M	14, 12, 13	22, C.W., C.W.
10	F	12, 9, 8	20, 18, 16
11	M	7, 6, 4	20, 16, 20
12	F	3, 2, 0	20, C.W., 20

(C.W. = completely wet)

Table II,1 Recordings of unforced Schirmer tear test in rheumatoid arthritic patients related to temperature and humidity. None of the patients had keratoconjunctivitis sicca.

the test was consistent for either hospital, and readings were consistently lower in the higher temperature and lower humidity of the rheumatic wards (Table II,1). It must be noted that reduction of measurable tear secretion by the unforced Schirmer test occurred after the patients had been in the rheumatic hospital for 12 hours or more. When the patients were tested just after their return to the wards normal readings were recorded.

The Schirmer I test was repeated every second night in each of these 12 patients for the next 20 days. There was no tendency for the patients to compensate by increased production of tears, i.e. the results remained consistently low. Furthermore, within three days of hospitalization, 10 of the patients complained that their eyes felt hot and dry towards early afternoon and within seven days of entering hospital all 12 had uncomfortable eyes.

Discussion

Sixty-five of 338 patients (20%) admitted to the Centre for Rheumatic Diseases, tested 12 hours or more after entering the hospital, had less than 15 mm. of wetting of Schirmer papers after 5 minutes. All of these patients were re-examined a week later at an ophthalmic outpatient department and were found to have normal Schirmer I tear tests. They did not have

keratoconjunctivitis sicca. The findings appear to be related to the differences in temperature and humidity between the two hospitals, the readings being consistently lower in the warmer and dryer atmosphere of the rheumatic centre. 12 patients were examined repeatedly over a period of 20 days. The results were reproducible. There was no improvement in the low readings during this time and symptoms of dry, hot or irritable eyes developed in all of the 12 patients within a week of entering the rheumatic hospital.

If the reduction in wetting of the filter paper, observed in 20 per cent of the ward patients, was due merely to increased evaporation in a warm, dry atmosphere, then would 12 hours have elapsed before the phenomenon was apparent? Furthermore, why should there be a selection of patients if the explanation lies in the purely physical process of evaporation? Emotional factors could have been responsible for an increased lacrimal secretion in these suspect patients until they settled down in the wards and began to produce their normal basal level of tears. If this explanation is correct then there must be a poor response to dry heat in some patients suffering from rheumatoid arthritis. If an equivalent number of normal control patients were subjected to the conditions that prevail in the rheumatic wards, then the incidence of reduced Schirmer I tear tests could be obtained. To date this experiment has proved to be impractical because of the cost and number of volunteers that would be necessary. It may be that

the paucity of lacrimal response to temperature and humidity occurs in rheumatoid arthritic patients who have some infiltration of the lacrimal glands but enough functioning tissue to prevent the development of keratoconjunctivitis sicca. Whatever the cause of this observation, the effect of temperatures over 72°F. and humidities below 40.5 is to produce 20 per cent false positive Schirmer I tear tests in rheumatoid arthritic patients.

The Forced Schirmer Tear Test

From what has been demonstrated above, it can be seen that some form of augmented Schirmer tear test is necessary when the diagnosis of keratoconjunctivitis sicca is suspected.

Schirmer (1903) anaesthetized the surface of the eye by instilling 4 per cent solution of cocaine in order to eliminate the stimulus caused by the insertion of the blotting paper, inserted a camel hair brush into the nostril and by rotating it caused reflex lacrimal gland secretion. He demonstrated that most normal patients wetted the strips of filter paper to 15 mm. in 2 minutes. Sjøgren (1935) substituted red litmus paper and ammonia fumes finding them to be as good as the nasal brush and less troublesome to the patient. Stock (1924), Isakowitz (1928) and Betch (1930) had also used ammonia but in none of their reports nor in Sjøgren's was the

strength of the solution stated. Ammonia solutions are normally at a strength of five per cent. However, it was noted during the first 6 months of this project that Schirmer tests augmented with a five per cent concentration of ammonia did not produce better results than those recorded by the Schirmer I method. Consequently, 25 patients who had produced less than 15 mm. of wetting in two minutes with five per cent ammonia and who did not have keratoconjunctivitis sicca were recalled and tested with a ten per cent solution over 5 minutes. Only three of the 25 patients had false positive tests with this method. Ten per cent ammonia solution held 6 inches from the patient's nose for 5 minutes was adopted, therefore, as the standard method for a Schirmer II tear test. In an attempt to further reduce error, reduced tear secretion was diagnosed only when the forced test was positive on two separate occasions.

Summary

Schirmer I tear tests are unreliable particularly when carried out in a warm, dry atmosphere. Forced Schirmer tests using ten per cent ammonia are satisfactory, reduced tear secretion being diagnosed when the test is positive on two separate occasions.

Symptoms and Signs in Keratoconjunctivitis Sicca

The diagnostic signs of keratoconjunctivitis sicca defined by Sjøgren (1933) are relatively simple to detect with a corneal microscope. However, the slit lamp is a piece of equipment that requires some expertise not normally acquired by a physician. Furthermore, not all rheumatology units are fortunate enough to have all of their patients examined by an ophthalmologist. Therefore, it seemed important to design a diagnostic index for keratoconjunctivitis sicca which might be of some use to the rheumatologist.

Fig.II,3 Lacrimal gland enlargement in Sjøgren's syndrome - patient No.46, appendix IX.

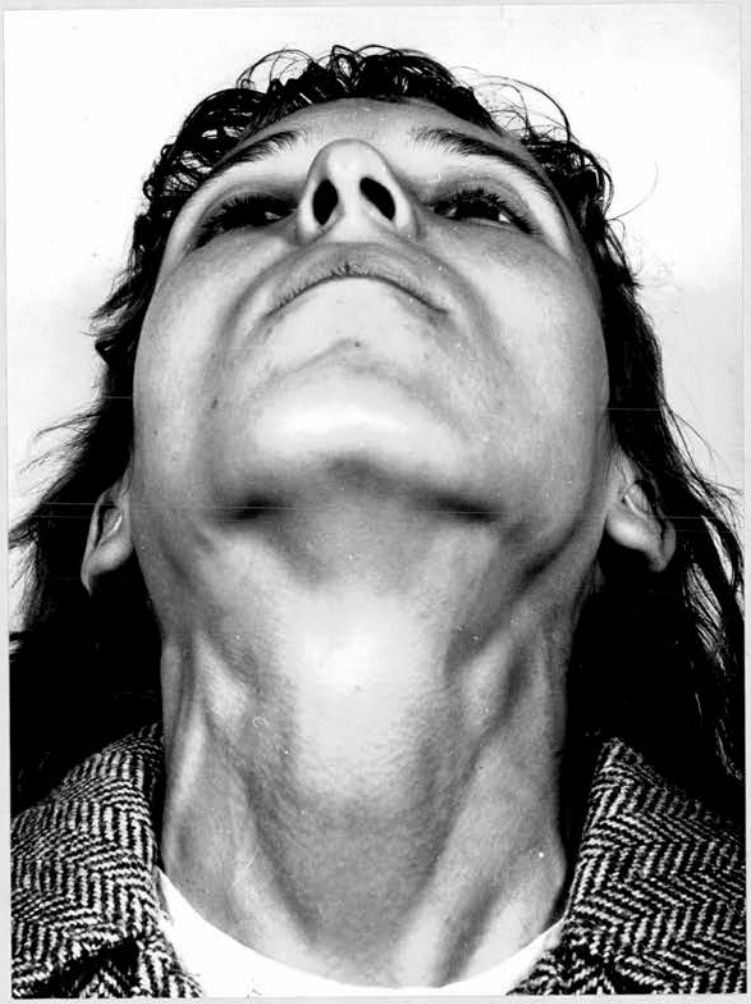


Fig.II,4 Salivary gland enlargement in Sjøgren's syndrome - patient No.50, appendix IX.

Fig.II,5 Lacrimal and salivary gland enlargement in Sjøgren's syndrome. Injection of conjunctival vessels and perilimbal injection readily detected - patient No.80, appendix IX.

The Keratoconjunctivitis Sicca Index

This section deals with the evolution and trial of a diagnostic index for keratoconjunctivitis sicca. The study was conducted in two phases.

Phase One

The symptoms, gross signs and slit lamp appearances of 40 patients not known to have Sjøgren's syndrome before examination, and 40 age and sex matched rheumatoid arthritic patients with no evidence of external ocular disease were tabulated. The patients had either received no topical treatment or none for at least 8 weeks before examination. Thus an attempt was made to exclude patients who may have benefited from topical therapy. Furthermore, patients like those depicted in Figures II,3,4 and 5 showing lacrimal and salivary gland enlargement were excluded from the study. Their appearances would have immediately drawn the physician's attention to the possibility of Sjøgren's syndrome and the idea of this investigation is to develop a system that will enable unsuspected cases to be diagnosed.

The list of the patients' symptoms was compiled by noting voluntary statements and by completing a questionnaire based on the prevalence of ocular complaints detected by Henderson (1950). Gross signs and slit lamp

TEN SYMPTOMS IN KERATOCONJUNCTIVITIS SICCA

	SYMPTOMS	Percentage Present
1.	Foreign body sensation	88.0
2.	Burning	75.0
3.	Tiredness with or without difficulty in opening the eyes	70.0
4.	Dry feeling with or without a poor response to physical or chemical irritants and emotion	65.0
5.	Redness	47.0
6.	Difficulty in seeing	40.0
7.	Itch	37.5
8.	Aches, soreness or pain	37.5
9.	Photosensitivity	25.0
10.	Excess of secretion, watery, ropy or film	22.5

Table II,2

SIGNS IN KERATOCONJUNCTIVITIS SICCA ON NAKED EYE EXAMINATIONS

(40 patients)

		Percentages
1.	Dilated bulbar conjunctival vessels, usually interpalpebral	77.5
2.	Mild pericorneal injection	52.5
3.	Photophobia	27.5
4.	Irregularity of corneal image	27.5
5.	Discharge - white and frothy, or yellowish and tenacious	25.0
6.	Dullness of conjunctiva and/or cornea	25.0
7.	Ptosis	15.0

Table II,3

Fig.II,6 showing macroscopic signs, discharge white, yellow and tenacious, dullness of conjunctiva, ptosis - patient No.5, appendix II.

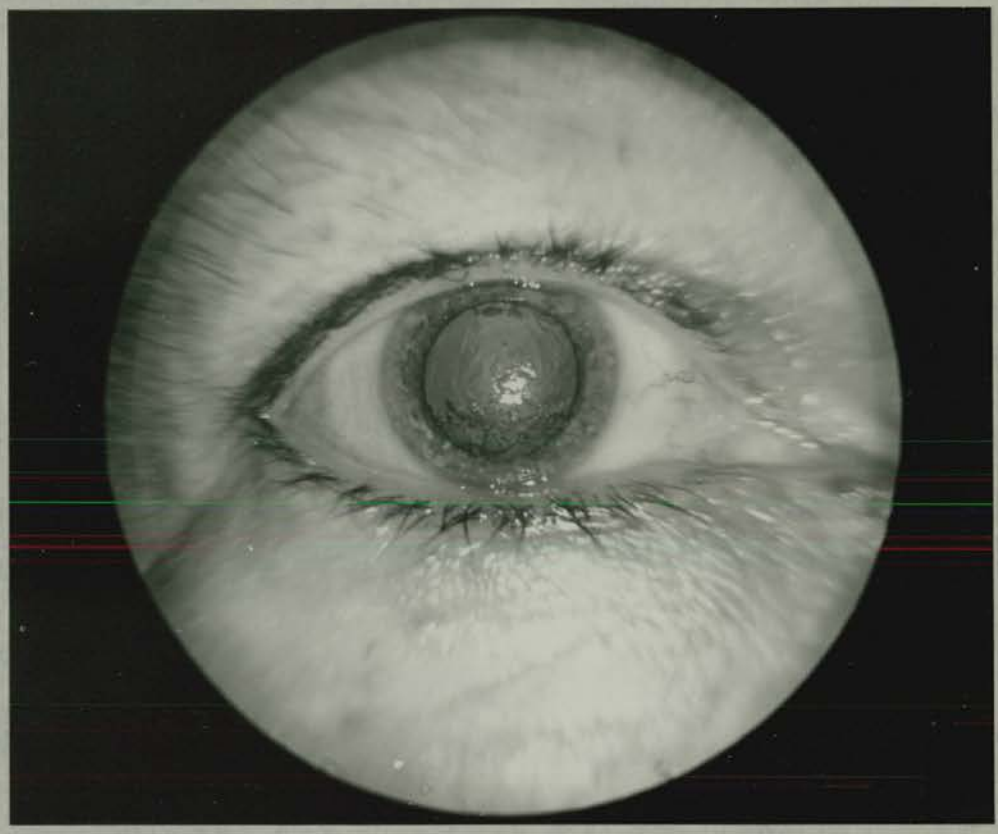


Fig.II,7 Macroscopic sign - irregularity of the corneal image. This photograph was taken following instillation of adrenaline 1:1000 and homatropine one per cent. The camera was focused on the anterior lens capsule and the cornea retro-illuminated - patient No.6, appendix II.

examinations were recorded where possible in the terminology used by Sjøgren (1933).

Phase Two

A prospective study of 100 rheumatoid arthritic patients who had not been examined previously by the author was carried out using the Index developed as a result of Phase One. The diagnosis was confirmed by the usual tear tests and slit lamp examinations.

Results

Tables II,2,3 and 5 show the results of the ophthalmological examinations in the 40 rheumatoid arthritic patients diagnosed as cases of Sjøgren's syndrome. Examples of some of the macroscopic signs listed in Table II,3 are shown in Figures II,6 and 7.

The prevalence of 10 symptoms and 7 gross signs in the Sjøgren group of patients can be compared with that in the control series of arthritic patients who did not have keratoconjunctivitis sicca. By weighting each of the 17 factors involved a "Keratoconjunctivitis Sicca Index" is produced (Table II,4 - see Appendix to Chapter II, page 251). A score of 24.4 $\pm$ 4.23 Standard Error was recorded in the Sjøgren group whereas a score of only 1.18 $\pm$ 0.71 Standard Error was

SYMPTOMS AND GROSS SIGNS	PRESENT +	ABSENT -
1	5	-1
2	5	0
3	4	0
4	5	0
5	3	0
6	2	0
7	3	-2
8	2	0
9	1	0
10	2	0
11	2	0
12	2	0
13	5	-2
14	3	-1
15	2	0
16	2	0
17	1	0
TOTALS		

TOTAL SCORE

CASE No. :

DIAGNOSIS :

Table II,4 Example of scoring system for diagnostic index. Patient No.1 total score 43, diagnosis keratoconjunctivitis sicca.

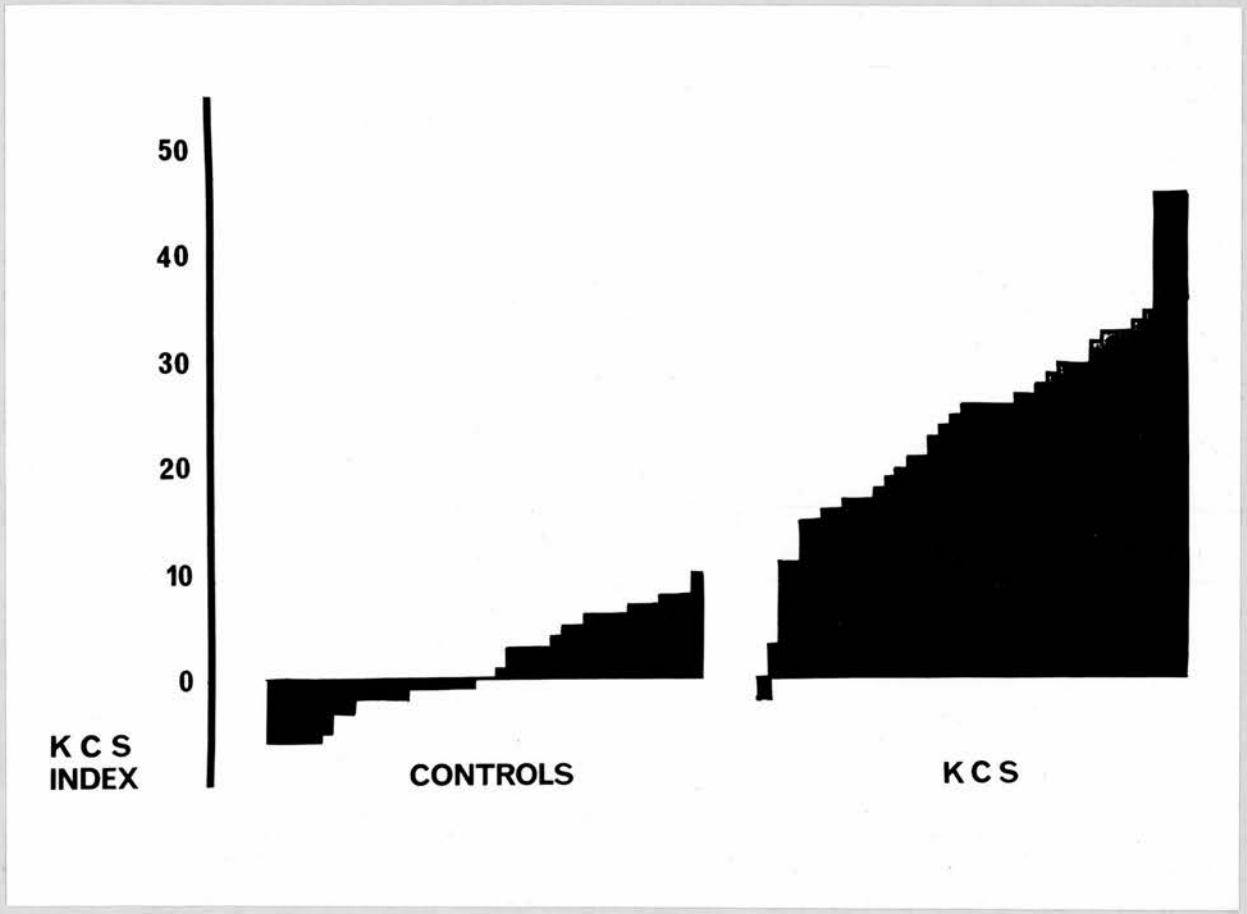


Fig.II,8 Histogram showing the dramatic difference between the control group of rheumatoid arthritic patients who had low index scores and those with keratoconjunctivitis sicca (K.C.S.) who had high scores. A score of 10 or more indicates K.C.S.

observed in the control series (Fig.II,8). Only two of the patients suffering from keratoconjunctivitis sicca had a score of less than 10.

Application of the Index to the 100 rheumatoid arthritic patients in phase two of the investigation resulted in a correct diagnosis of keratoconjunctivitis sicca in 8 patients (8%). Another patient who had Sjøgren's syndrome was missed by the diagnostic Index but no false positive diagnoses were made.

Discussion

Sjøgren (1933) described in detail all of the clinical criteria necessary for a diagnosis of keratoconjunctivitis sicca. However, there have been considerable variations in the reported prevalence of both subjective and objective phenomena in the disease partly as a result of selection of cases, natural remissions and treatment (Gifford, Puntenney and Bellows, 1943; Henderson, 1950; Bloch, Buchanan, Wohl and Bunim, 1965). The data analysed in this study was collected from new and untreated cases of Sjøgren's syndrome with a view to composing a system whereby the physician could diagnose keratoconjunctivitis sicca.

The prevalence of 10 symptoms and 7 gross signs in Sjøgren's syndrome, confirmed by forced tear tests and

slit lamp examination, were recorded. When the 17 factors were weighted to favour a diagnosis of keratoconjunctivitis sicca and applied to 40 control patients, it was seen that a highly effective diagnostic index had been developed. Thus a score of 10 or more almost certainly indicates that the patient has keratoconjunctivitis sicca. Conversely, only two patients with a score of less than 10 had dry eyes. The value of this "Keratoconjunctivitis Sicca Index" was tested in a prospective study of 100 rheumatoid arthritic patients who, in addition, were examined by Schirmer II tear tests and the corneal microscope. Nine patients (9%) had keratoconjunctivitis sicca, 8 being diagnosed initially by the Index. Furthermore, the diagnostic Index did not result in any false diagnoses of keratoconjunctivitis sicca.

In conclusion, the "Keratoconjunctivitis Sicca Index" is a useful screening technique that can be applied by the physician. At present no erroneous diagnoses of keratoconjunctivitis sicca have been made and only one patient in 100 rheumatoid arthritics has been missed.

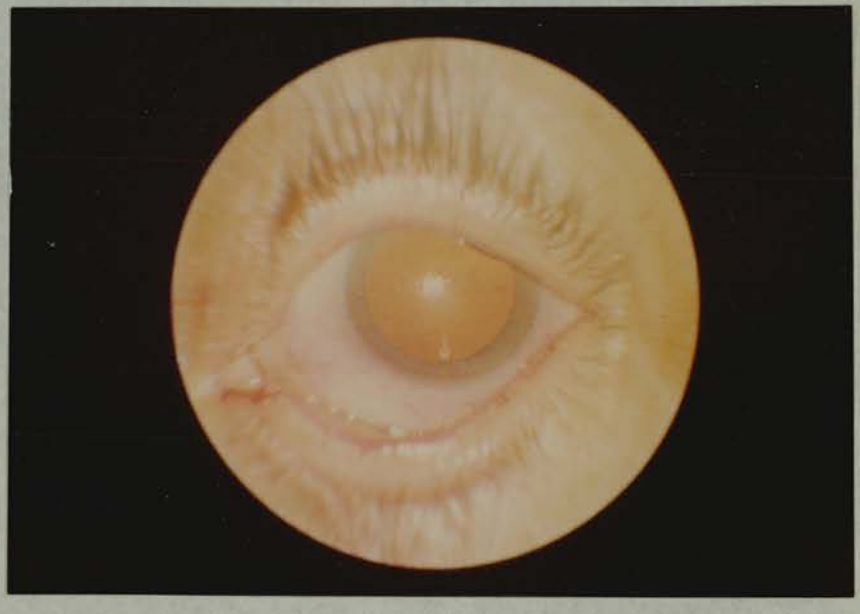
BIOMICROSCOPY changes in KERATOCONJUNCTIVITIS SICCA. (40 patients)

Before bengal rose dye	
Conjunctiva	Percentage
Dilated interpalpebral vessels	77.5
Mild pericorneal injection	52.5
Mucus in conjunctival sac	25.0
Cornea	
Viscous tear film	50.0
Mucous threads in tear film	25.0
Filaments attached to cornea	10.0
Large mucous threads attached to cornea	5.0
After bengal rose dye	
Conjunctiva	
Triangular interpalpebral staining	100.0
Marked	15.0
Intermediate	65.0
Scattered	20.0
Mucous attached to conjunctiva	20.0
Cornea	
Punctate staining - including staining of pits	40.0
Mucous threads in tear film	25.0
Filaments attached to cornea	20.0
Large mucous threads attached to cornea	5.0
Fluorescein staining	
Cornea	30.0

Table II,5 Microscopic examination of 40 patients with keratoconjunctivitis sicca.

Fig.II,9 Scattered staining of conjunctiva and cornea with bengal rose dye. Adrenaline 1:1000 instilled before photography to enhance appearance of vital staining - patient No.22, appendix II.

Fig.II,10 Intermediate staining of conjunctiva and cornea - patient No.20, appendix II.



Fig,II,11 Marked staining of conjunctiva and cornea. Photographic technique as in Fig.II,7.

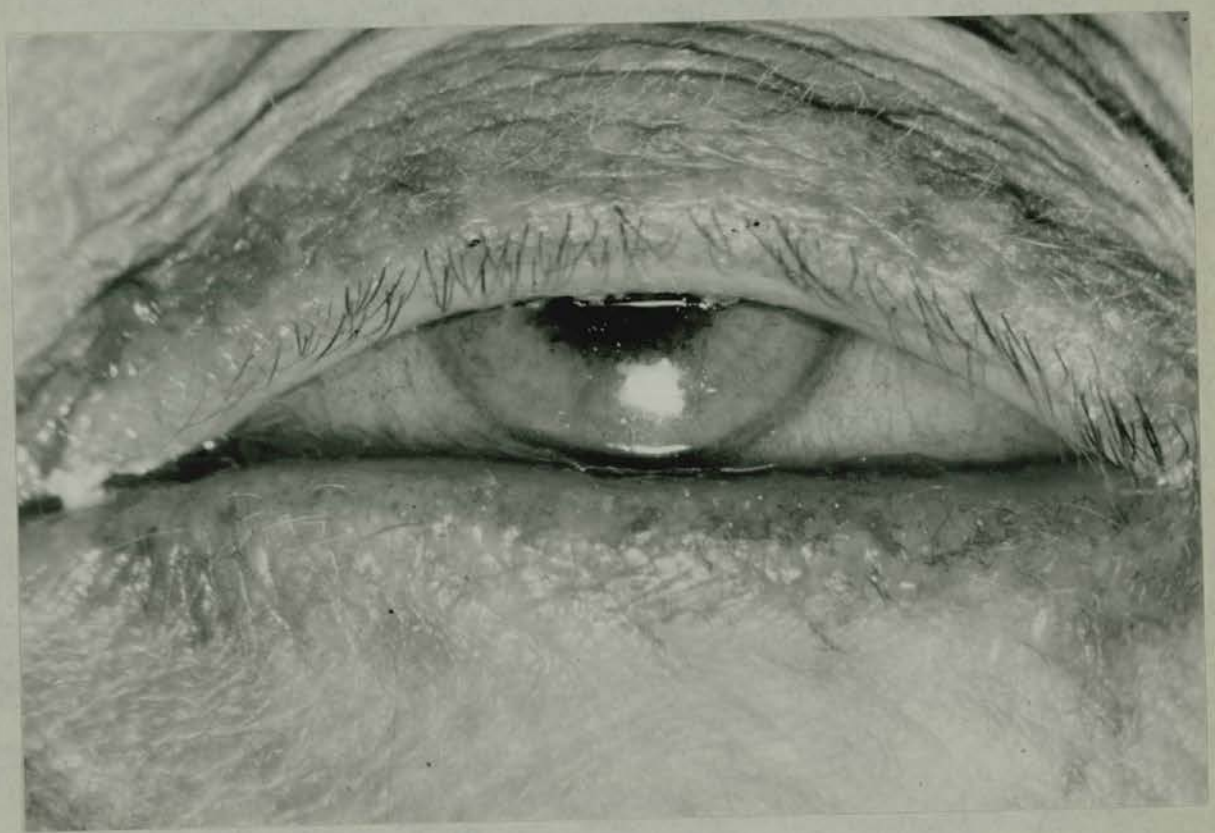


Fig.II,12 Mild pericorneal injection and viscous tear film. This patient also had ptosis. No.12, appendix II.

Fig.II,13 Mucus in the tear film. Patient No.19, appendix II.

Fig.II,14 Slit lamp photograph of large mucous shreds attached to cornea. Patient No.11, appendix II.

Slit Lamp Examinations in Keratoconjunctivitis Sicca

Results

Table II,5 shows the frequency of ocular signs in the 40 patients suffering from untreated keratoconjunctivitis sicca. The results of the examinations were recorded before and after the instillation of one per cent bengal rose and two per cent fluorescein. Interpalpebral staining of the conjunctiva with bengal rose was present to some extent in every case (Figs.II, 9,10 and 11) and dilated interpalpebral conjunctival vessels in 77.5 per cent. Mild pericorneal injection and a viscous tear film were the next commonest features present in some 50 per cent of cases (Fig.II,12). Punctate staining usually of the lower third to two thirds of the cornea with bengal rose and fluorescein was a feature of 30 to 40 per cent and was rather more common than mucus in the tear film (Fig.II,13) or attached to the surface of the cornea (Fig.II,14). Large mucous threads were distinguished from small corneal filaments for the purposes of this study although it is recognised that filaments are largely mucus (v.i.). Thus tiny corneal filaments were observed in 20 per cent of cases following instillation of bengal rose dye whereas large mucous threads were present in about 30 per cent. Moreover, corneal filaments were never present in the absence of

mucus in the tear film or punctate staining of the cornea with rose bengal.

Sjøgren (1933) recognised tiny circular grey facets and pits especially in the lower two thirds of the cornea. These have been designated "pits" in Table II,5. Occasionally the lesions extend through Bowman's membrane into the anterior stroma. When the pits have been resurfaced with epithelium dye is not taken up and they are more difficult to distinguish.

Fluorescein patterns of staining varied considerably, isolated punctate erosions, linear confluent erosions, widespread punctate erosions and punctate keratitis. Some of these patterns suggested the possibility of adenovirus or early herpetic infections and stimulated some of the investigations described in Chapter VIII.

Discussion

The possible causes of the ocular signs in Sjøgren's syndrome require careful consideration. Conjunctival hyperaemia, particularly of the interpalpebral vessels, was a prominent feature of 77.5 per cent of the patients described in this series (Table II,5). Although this is constantly referred to in series of patients suffering from Sjøgren's syndrome, Holm (1949) considers that it is not pathognomonic of the disease but rather indicative of superimposed infection. Certainly the

prevalence of pathogenic bacteria in the conjunctival sacs of untreated keratoconjunctivitis sicca patients is higher than in arthritics with no evidence of external eye disease (Cahpter VIII). On the other hand, the overlying conjunctiva always shows evidence of dessication and cell necrosis in that rose bengal stain is constantly present. It seems likely that some degree of hyperaemia is typical of the disease but that it may be enhanced by bacterial infection.

As a result of reduced tear secretion and increased goblet cell formation in the conjunctiva (Sjøgren, 1933; Klein, 1949) mucus accumulates in the eyes of patients suffering from keratoconjunctivitis sicca. Thus the tear film increases in viscosity (Klein, 1949) and its normal firm adherence to the cornea (Fischer, 1928) is increased. Mucous shreds may be detected either lying on the surface of the cornea and conjunctiva or attached to their surfaces (Table II,5). There is convincing evidence that a proportion of the corneal changes in keratoconjunctivitis sicca is brought about by the interaction of the abnormally tenacious mucus and the already diseased cornea. Sjøgren, 1933, examined autopsy material from two of his 19 cases and detected fine threads of superficial corneal "fibrillae" attached to the superficial part of an epithelial cell or several cells. He did not examine the material in any greater detail. However, Hess (1892) and Nuel (1892, 1893) had stated that the filaments in filamentary keratitis arise from a triangular elevation of epithelium

which is elongated into a spiral containing a core of mucus. Nuel (1892) suggested that the cell-like structures sometimes seen in the terminal expansion of a filament are conjunctival squames picked up by the aggregation of mucus. Weskamp (1956) observed what he regarded as an eruption of gelatenous degenerated stromal material carrying epithelial cells with it.

The introduction for clinical use of one per cent alcian blue by Norn (1962, 1963) helped to clarify the nature of the shreds and filaments. Alcian blue stains choroidoitin - and mucotin - sulphate complexes in mucin. Norn detected intense staining of the cornea in keratoconjunctivitis sicca indicating that the filaments consisted largely of mucin. The central core of the filament stains densely with this vital dye and Jones and Coop (1965) could not detect any cells or cell-like structures except at its base. Furthermore, Norn (1969) was able to demonstrate that the rate of flow of mucus towards the medial canthus was reduced in keratoconjunctivitis sicca. Thus, it is postulated that the abnormal quantities of mucus, produced by the goblet cells in the conjunctiva of keratoconjunctivitis sicca patients, are deposited on the surface of the cornea, possibly on cells already damaged by dessication. As the mucus builds up it may form filaments or may tear off corneal cells either singly or in strands. In this context it is important to recall that none of the patients described in the preceding pages had filaments in the absence of mucus shreds or bengal rose staining of the corneal epithelium.

Summary

The prevalence of symptoms, macroscopic and microscopic signs in 40 new cases of Sjøgren's syndrome are described. Consideration of the symptoms and gross signs in relation to a control series of 40 arthritic patients with no evidence of keratoconjunctivitis sicca led to the development of a "Keratoconjunctivitis Sicca Index" for use by the physician. A score of 10 or more indicates that the patient has keratoconjunctivitis sicca. The Index was evaluated in a series of 100 rheumatoid arthritics and found to be a highly successful screening procedure.

The possible causes of some of the microscopic changes in keratoconjunctivitis sicca, conjunctival hyperaemia, excess mucus, corneal filaments and corneal staining patterns are discussed.

Future Developments

Lysozyme Tests

Various methods for measuring the protein content of tears have been in use for several years. Using a viscometric technique, Meyer (1948) showed that the lysozyme concentration was reduced in keratoconjunctivitis sicca. His observation was confirmed by others using electrophoresis (Regan, 1950; McEwen and Kimura, 1955) and by Erickson (1955) who also demonstrated reduced lysozyme content in other ocular conditions such as paralimbal ring keratitis in systemic lupus erythematosus. Furthermore, Minton (1965) observed less lysozyme in the tears of patients living in polluted atmospheres than in those living in clean air conditions and Erickson, Matlon and Berg (1959), Sapse, Bonavida, Stone and Sercarz (1968) in older age groups with eye complaints. It appears, therefore, that many factors must be considered before a diagnosis of keratoconjunctivitis sicca can be made on the basis of reduced lysozyme concentration. However, Thygeson and Kimura (1963) showed that a decrease in concentration of lysozyme may precede all other evidence of keratoconjunctivitis sicca. The advent of a new technique - the agar diffusion lysozyme test (Bonavida and Sapse, 1968) with its increased degree of accuracy presents exciting prospects for the future. Lysozyme acts selectively on the cell walls of the micrococcus

Lysodeikticus. The effect of this action can be measured by the zone of lysis produced round an antibiotic disc in an agarose plate. Van Bijsterveld (1969) applied this method to patients suffering from keratoconjunctivitis sicca and claimed that it was more accurate than the Schirmer I tear test or staining with rose bengal dye. However, he did not compare it with a combination of the Schirmer II test and rose bengal staining. Nevertheless, the agar diffusion lysozyme test must be thoroughly evaluated by future observers. In this context it will be interesting to examine the lysozyme content in the tears of those rheumatoid arthritic patients who consistently produce very few tears in a warm, dry atmosphere.

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CHAPTER XII

OBSERVATIONS ON LACRIMAL GLAND HISTOLOGY

The histology of Sjøgren's syndrome has been studied at post mortem either in single cases or in series of fewer than 8 patients (Sjøgren, 1933; Bruce, 1941; Holm, 1949; Allington, 1950; Bohm, 1950; Ellman, Weber and Goodier, 1951; Hass, 1951; Reader, Whyte and Elmes, 1951; Morgan and Raven, 1952; Cardell and Gurling, 1954; Szanto, Farkas and Gyulai, 1957; Funatsu and Eguchi, 1957; Bucher and Reid, 1959; Bain, 1960; Bloch, Buchanan, Wohl and Bunim, 1965). Lacrimal and salivary glands, mucous glands of the respiratory tract, mouth and upper oesophagus are typically involved. However, most of the observations on lacrimal gland structure were recorded in severe cases of keratoconjunctivitis sicca (K.C.S.) and in patients who had died as a result of the severe complications of arthritis or another connective tissue disease. The primary purpose of this study was to investigate the histology of the lacrimal gland in patients currently suffering from K.C.S.

Patients with K.C.S. and xerostomia but no evidence of connective tissue disease may be classified as suffering from the 'sicca syndrome' (Chapter I). Lacrimal gland tissue from 'sicca syndrome' patients was also studied to determine any features that may differentiate the disease from Sjøgren's syndrome.

Reduced Schirmer II tear tests (Chapter II) may be demonstrated in a number of rheumatoid arthritic

patients who have no evidence of punctate or filamentary keratitis. For study purposes, these patients may be regarded as 'possible' K.C.S. and they are the subject of extensive clinical and serological examination reported in Chapters V and VI. Lacrimal gland biopsies were obtained from a sample of patients with 'possible' K.C.S. in an attempt to elucidate the validity of this classification.

Systemic corticosteroid or adrenocorticotrophic hormone therapy may be effective in controlling acute exacerbations of Sjøgren's syndrome (Manschot, 1961) and, therefore, may be expected to modify the histological appearances of the lacrimal gland. Thus particular note was taken of a history of systemic steroid treatment.

TABLE III,1

Clinical Condition	No. of Patients	Age (years)		Keratoconjunctivitis Sicca	
		Mean	Range	Definite	Possible
Rheumatoid Arthritis	24	61.4	52-80	18	6
Sicca Syndrome	4	68.5	55-81	4	-
Biliary Cirrhosis	1	67.0	-	1	-
Group Studied Post Mortem					
Coronary disease 5					
Diabetes Mellitus 2	10	64.0	32-76	-	-
Breast Cancer 2					
Drug Overdose 1					

Table III,1

MATERIALS AND METHODS

Patients Studied

Biopsies of the lacrimal gland were obtained from 29 female patients suffering from definite (23) or possible (6) keratoconjunctivitis sicca. In addition, the lacrimal glands of 10 age matched patients, who had died from a variety of medical disorders not related to rheumatoid arthritis or connective tissue disease and who had no history of ocular disease, were examined post mortem.

The diagnosis of rheumatoid arthritis, the clinical and laboratory methods referred to in the results and discussion are detailed in Chapters V and VI.

Lacrimal Gland Biopsy Technique

Since removal of part of the palpebral portion of the lacrimal gland would entail disruption of the excretory ducts, the orbital segment was chosen for biopsy. Each patient understood the reason for the operation and that it was to be carried out on a voluntary basis. Following infiltration with local anaesthesia, two per cent lignocaine without adrenaline, the skin was incised for a distance of 1.5 cm. from the lateral orbital tubercle towards the medial canthus, 0.5 cm. below and parallel to the superior orbital margin.

Underlying fascia and muscle were separated by blunt dissection until the smokey blue periorbital membrane was identified closely attached to the rim of the orbit. Incision of the periorbital membrane resulted in protrusion of periorbital fat which was easily replaced with a probe. Two into three toothed forceps were inserted into the superior lateral angle of the orbital roof and a piece of lacrimal gland tissue extracted through the gap in the periorbital membrane, severed with scissors and placed in formal saline. The skin wound was closed with three interrupted black silk sutures, no haemostatic or subcuticular stitches being necessary. Eyelid appearances returned to normal within 14 days and no complications were encountered. Histology was studied on 6 μ thick paraffin sections stained by haematoxylin and eosin. Considerable care was taken to present the pathologist with no information regarding the source of the lacrimal tissue.

Results

Table III,1 shows the range of disorders from which the patients were suffering. Two of the 24 rheumatoid arthritic patients also had systemic lupus erythematosus and three of the four 'sicca syndrome' group had osteoarthritis. All of the 6 patients with 'possible' K.C.S. were suffering from rheumatoid arthritis.

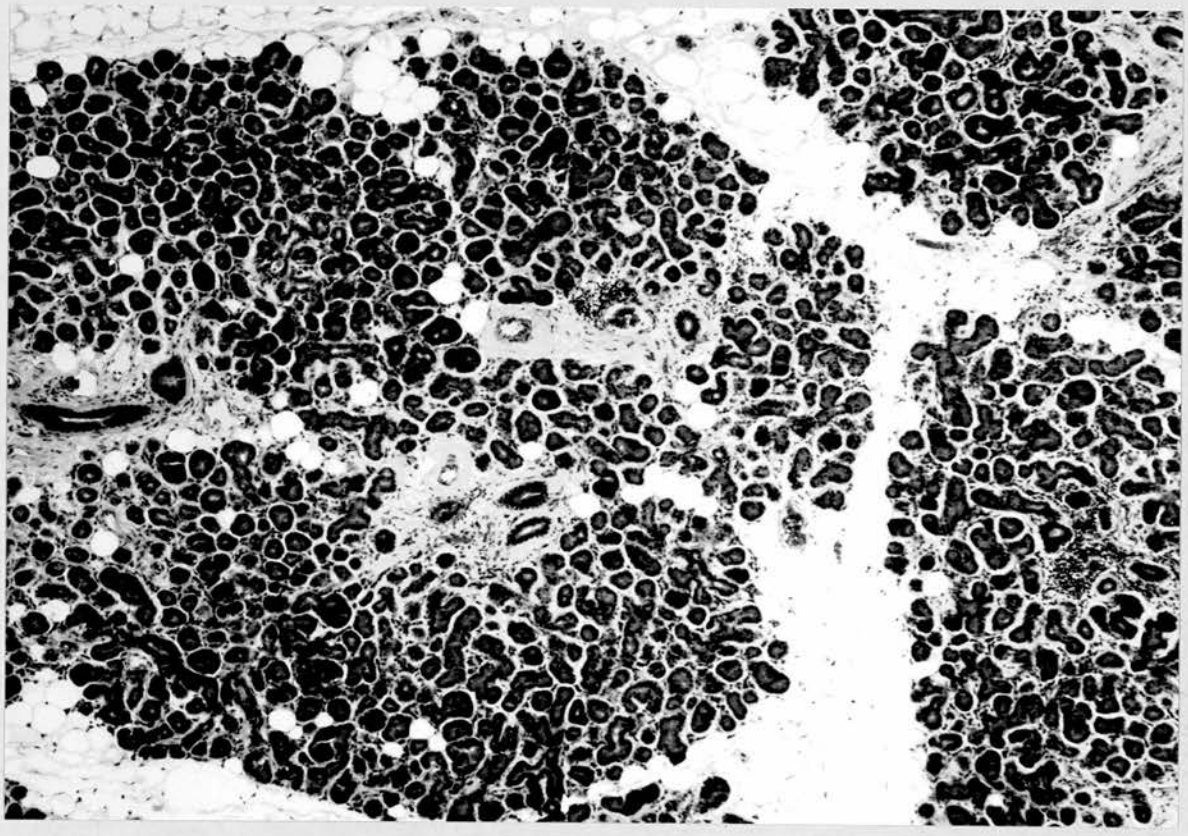

Fig.III,1 Lacrimal gland from a post mortem specimen of a 55 year old male patient who died of coronary disease. The normal architecture of the lobules, ducts and acini should be compared with those in Fig.III,6 where lobular destruction is evident.

(X4 magnification)

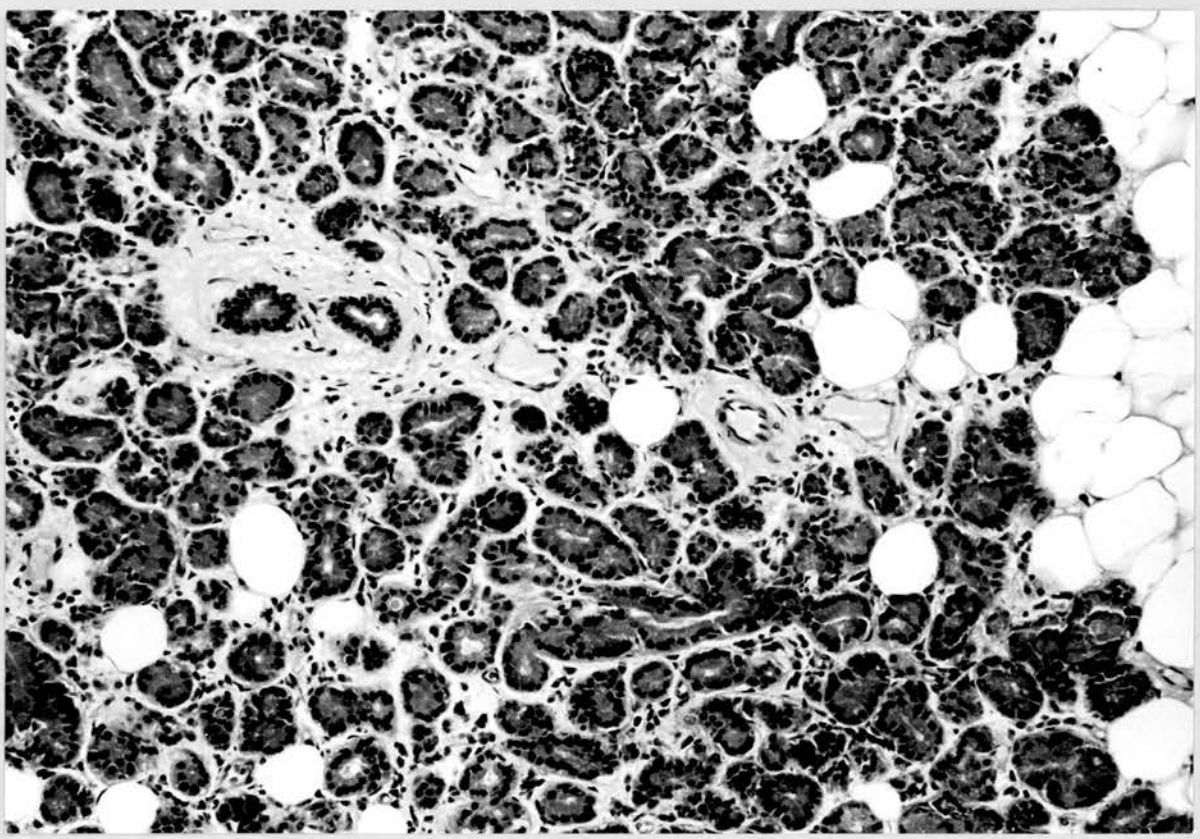

Fig.III,2 X10 magnification of section of the normal lacrimal gland shown in Fig.III,1. Fat spaces are interspersed among acinar tissue.

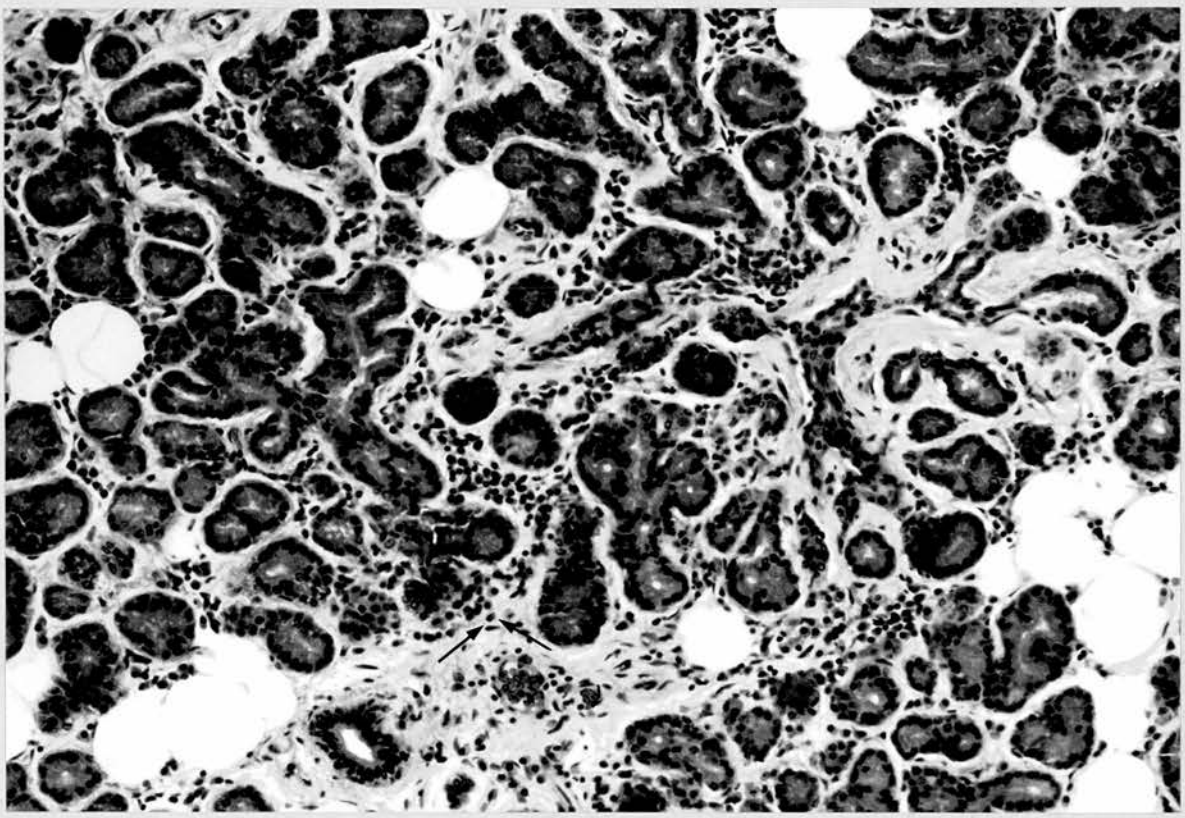

Fig.III,3 Normal lacrimal gland from a post mortem specimen of a 74 year old female patient who died as a result of secondaries from breast cancer. Plasma cells (arrowed) are frequently noted in normal specimens (X10 magnification).

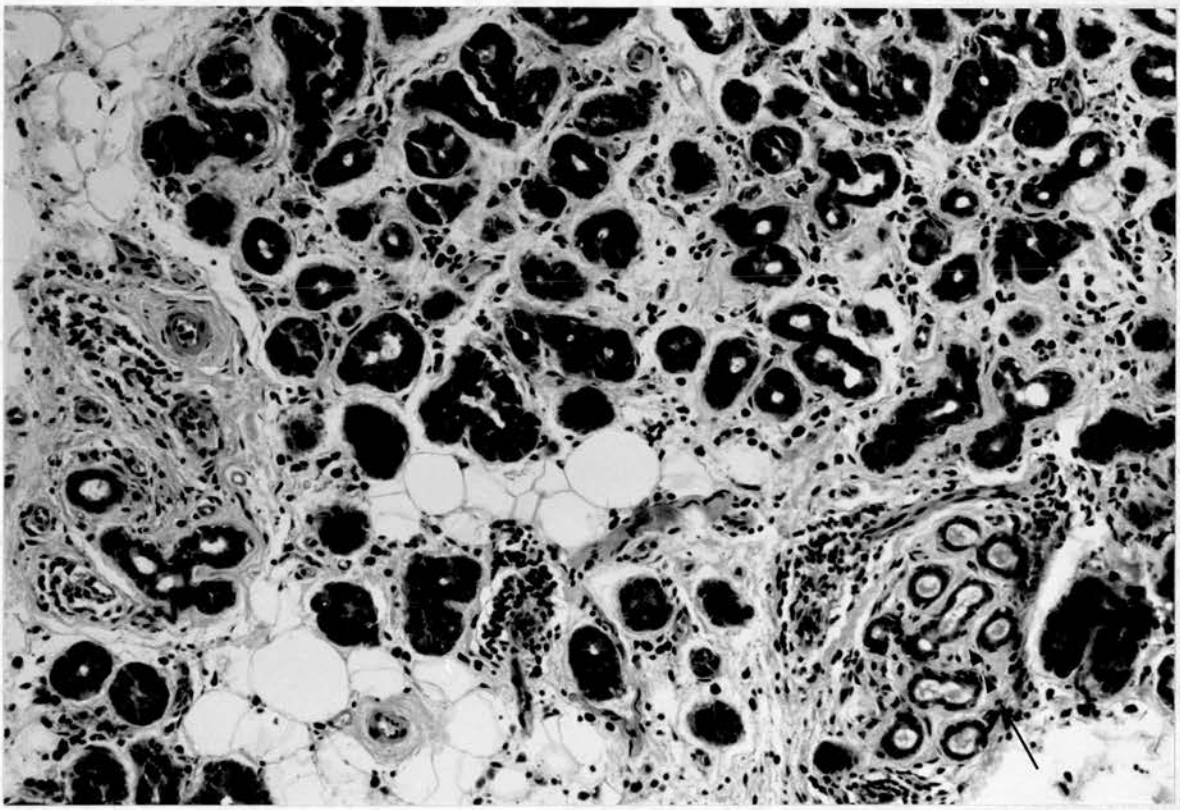

Fig.III,4 Biopsy specimen showing mild chronic inflammation. Early intralobular fibrosis is present but the main feature is the abnormal arrangements of the ducts (arrowed).

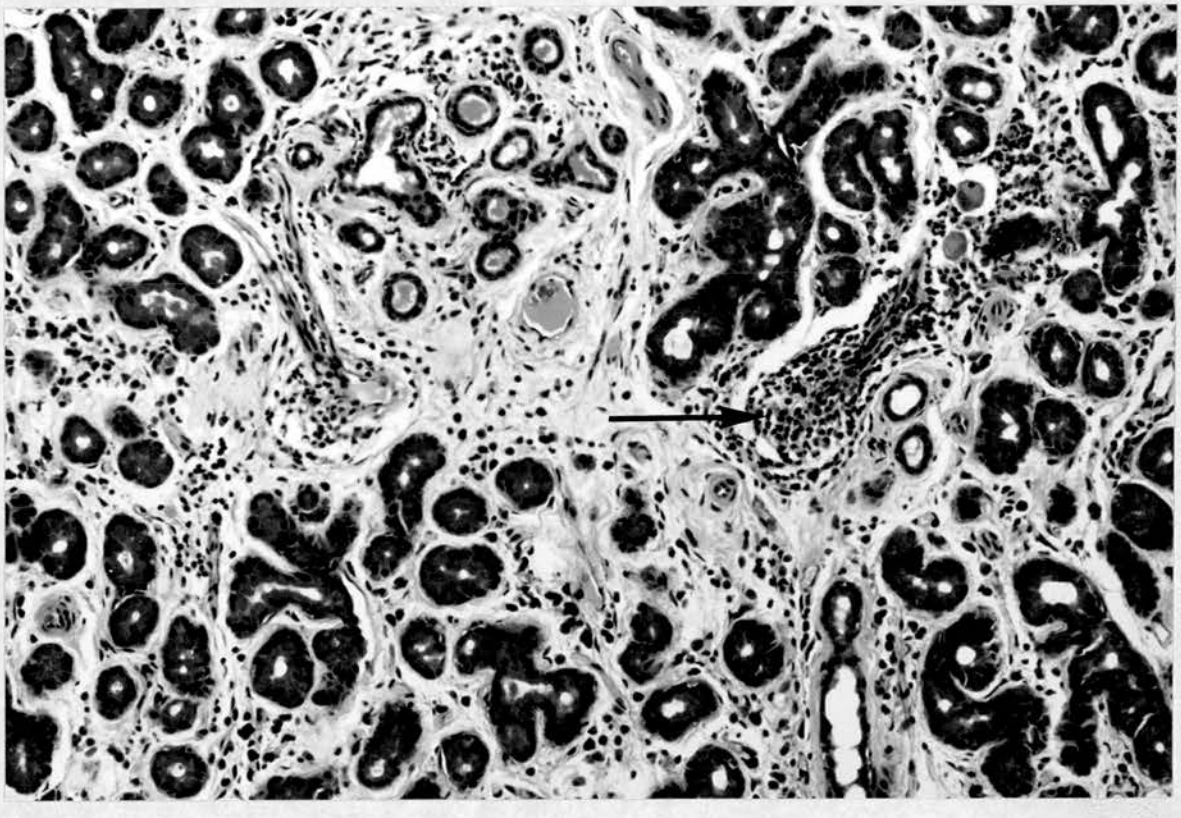

Fig.III,5 Mild chronic inflammation showing abnormal duct arrangement and infiltration with lymphocytes (arrowed). There does not appear to be any damage to the acinar epithelium.

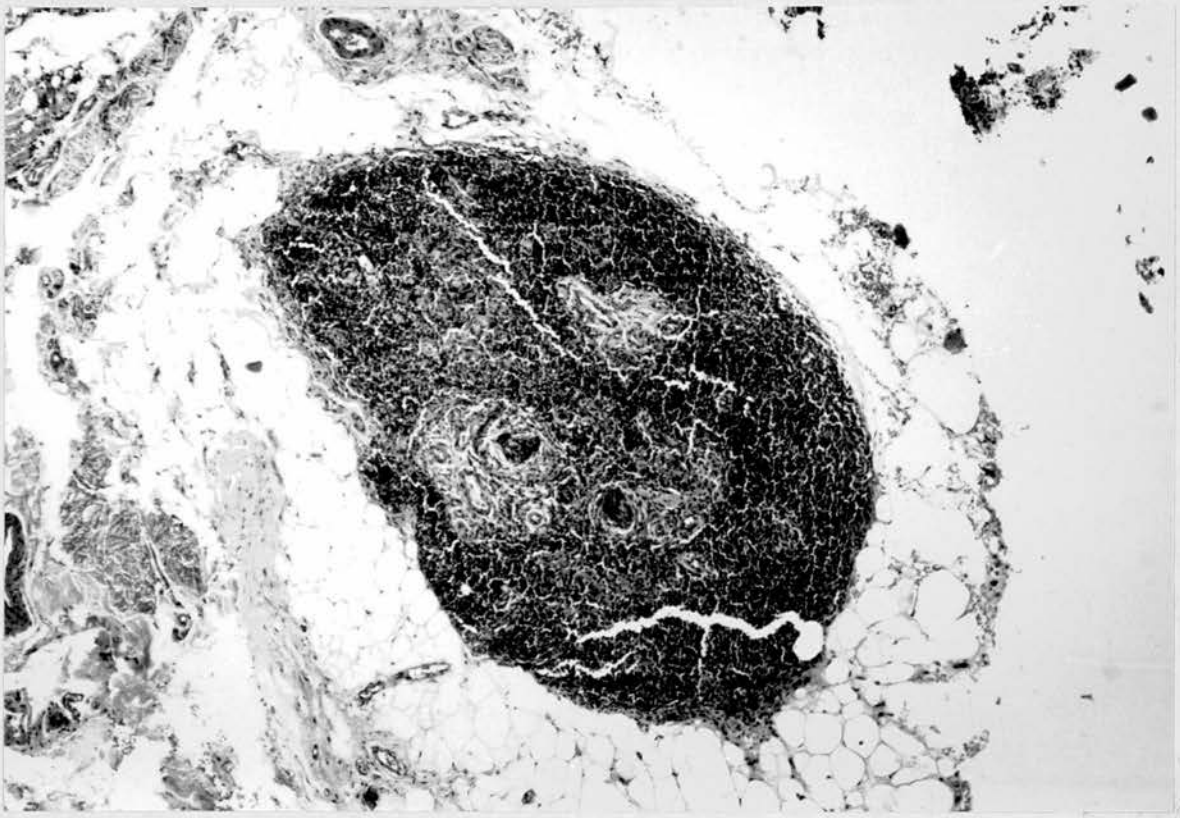

Fig.III,6 X4 magnification of an example of severe chronic inflammation. Themain feature is the destruction of the normal lobular pattern compared with that seen in Fig.III,1.

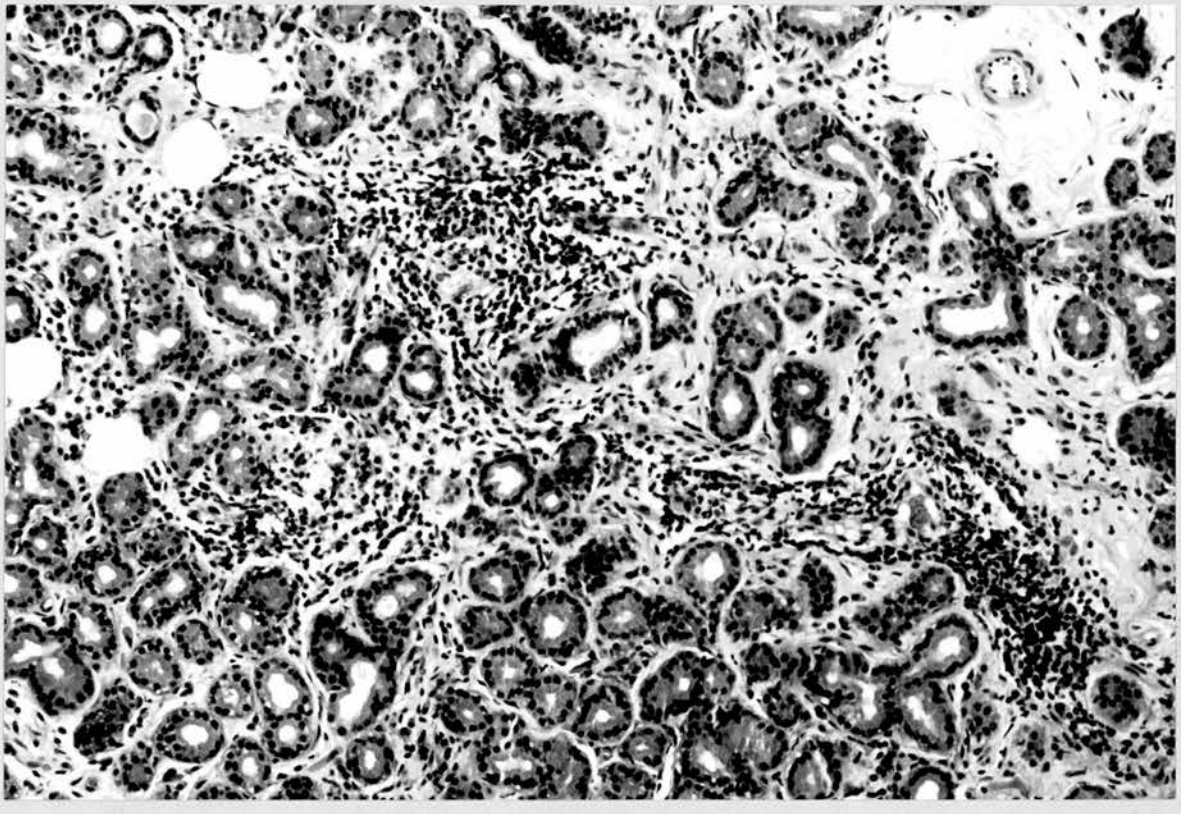

Fig.III,7 Dense aggregates of lymphocytes are present in severe chronic inflammation and acinar atrophy is evident in selected fields.

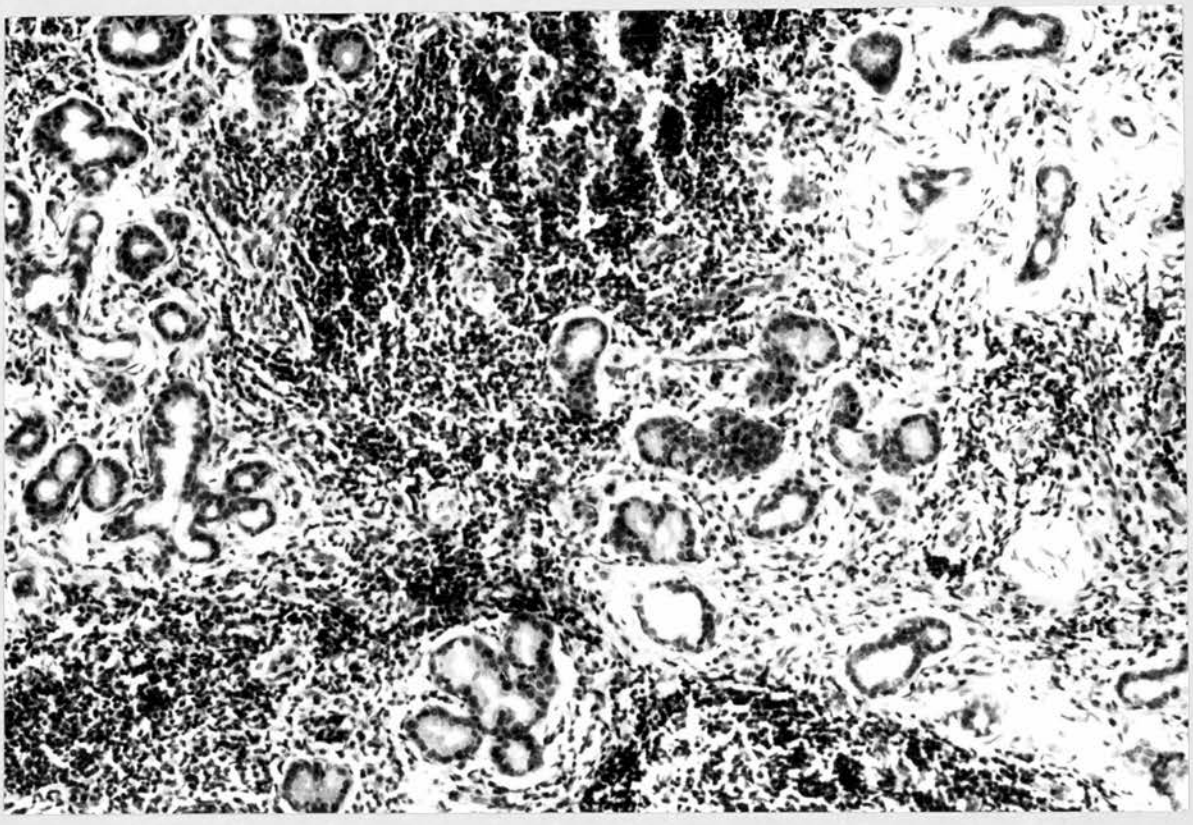

Fig.III,8 Severe chronic inflammation showing the most severe degree of acinar atrophy acceptable for this grade of specimen.

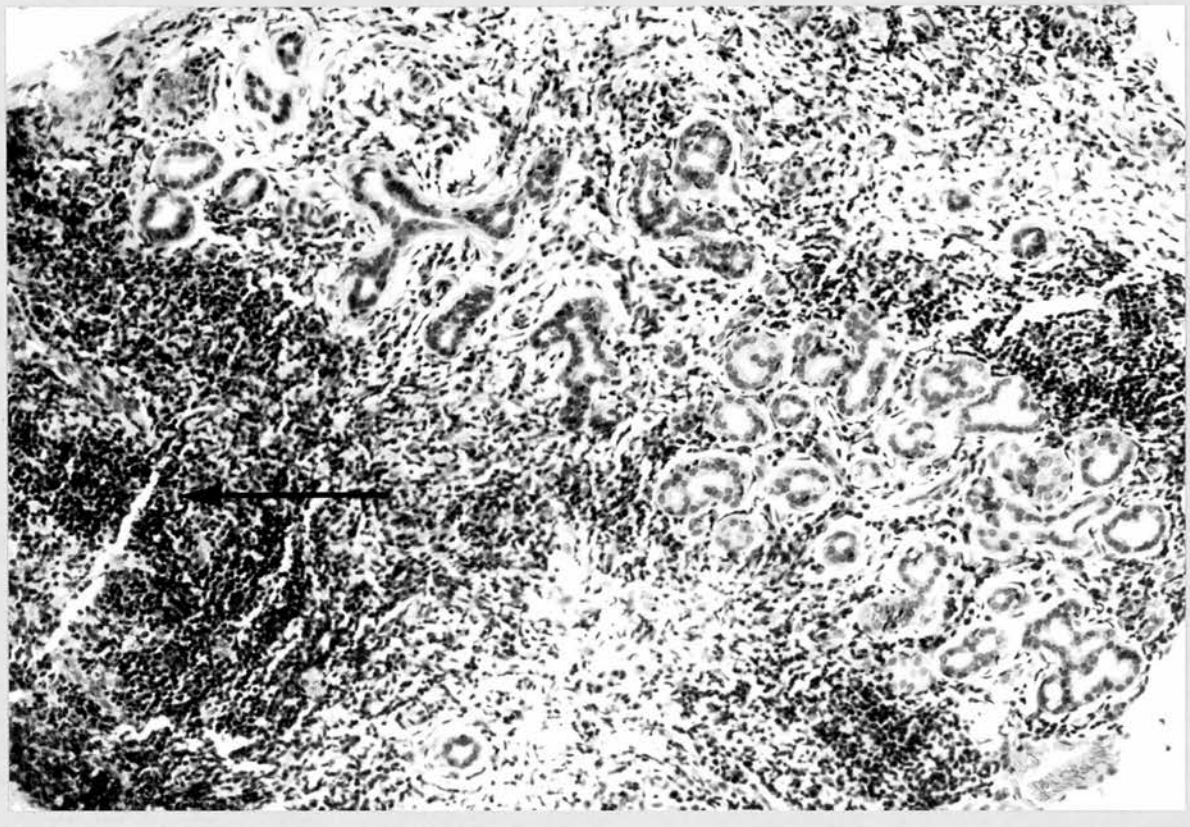

Fig.III,9 Lymphocytic infiltrations may reach a peak in severe chronic inflammation with the formation of lymph follicles and germinal centres (arrowed).

There was no significant difference in the mean age and age range of the groups studied.

Four distinct histological stages were recognised in this study.

Stage 0 - normal lacrimal gland.

The lacrimal gland consists of tubulo racemose tissue with short branched tubules and masses of lobules interspersed with fat (Figures III,1 and 2). Plasma cells are a normal feature of the interacinous and interlobular connective tissue (Figure III,3); lymphocytes are less frequently detected.

Stage I - mild chronic inflammation

The salient features in these cases are abnormal arrangement of the ducts, mild lymphocytic infiltration and slight intralobular fibrosis (Figures III,4 and 5).

Stage II - severe chronic inflammation

Total destruction of the normal lobular pattern (Figure III,6), dense infiltration with lymphocytes, aggregating into lymph follicles (Figures III, 7 and 9) and progressive acinar atrophy (Figures III, 7 and 8) are the features of this grade.

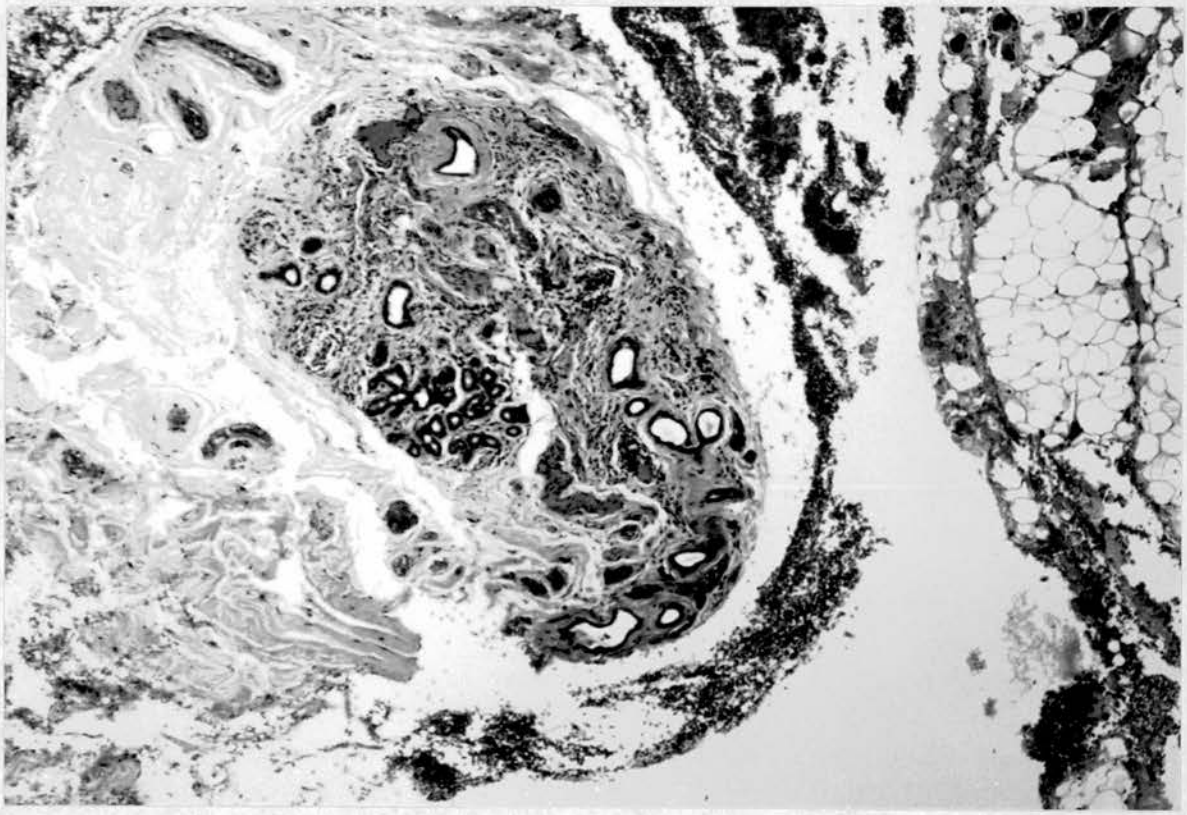

Fig.III,10 Late stage of lacrimal gland involvement in K.C.S. (X4 magnification). The total destruction of lobular pattern is evident but there is a reduction in the cellular infiltrate (compare Figs.III,1 and 6) and an increase in the acellular fibrous tissue.

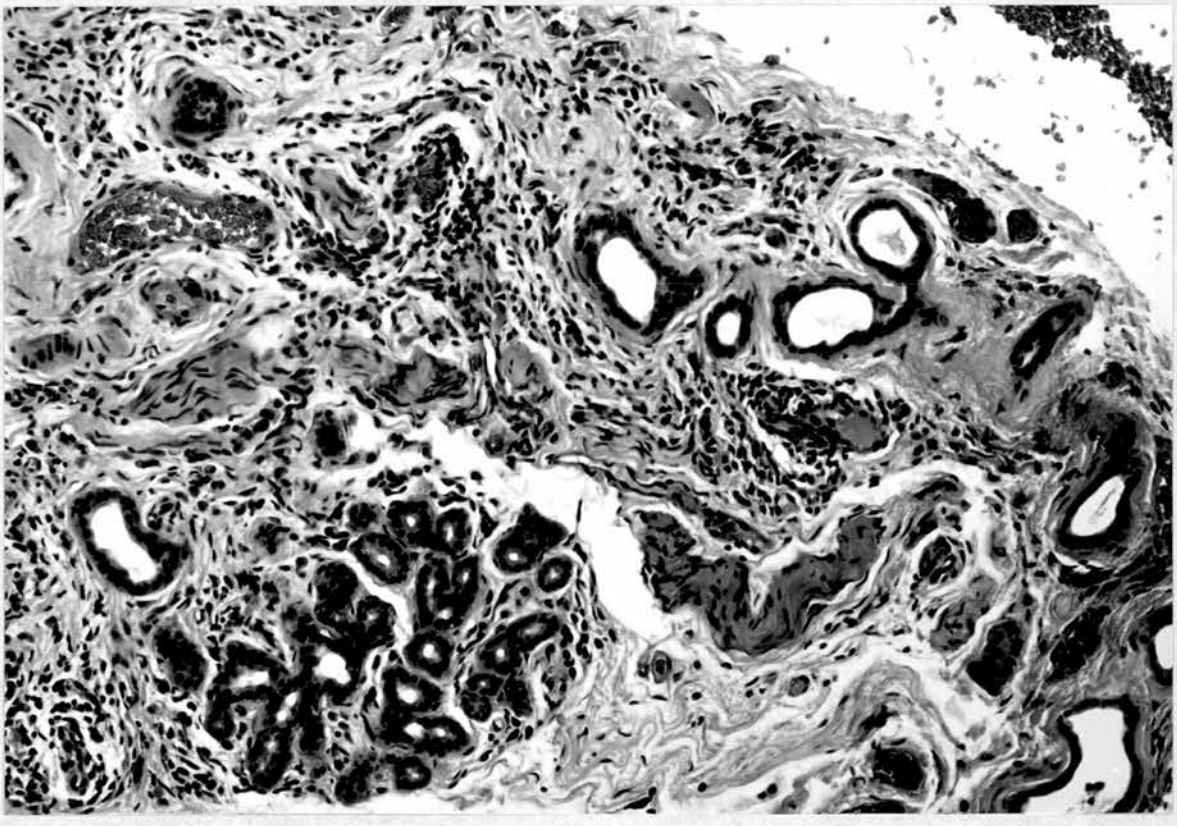

Fig.III,11 X10 magnification of the section shown in Fig.III,10. The dense fibrous tissue, few remaining acinar cells and marked atrophy are readily recognised features.

TABLE III,2

Clinical Condition	No. of Patients	Histology of Lacrimal Gland			
		0	I	II	III
Rheumatoid Arthritis with K.C.S.	18	1	-	9	8
Rheumatoid Arthritis with 'possible' K.C.S.	6	6	-	-	-
Sicca Syndrome	4	-	1	2	1
Biliary Cirrhosis	1	-	1	-	-
Control Patients	10	10	-	-	-

Table III,2

TABLE III,3

No. of Patients	Duration of K.C.S. (years)	Histology			
		0	I	II	III
4	0-1	-	1	3	-
4	1-3	1	-	3	-
3	3-5	-	-	-	3
4	5-7	-	1	3	-
8	7+	-	-	2	6
Total 23		1	2	11	9

Table III,3

Stage III - late results of inflammation

The cellular infiltrate is reduced and acellular fibrotic tissue is increased. Few acinar cells remain (Figures III,10 and 11).

All of the post mortem specimens and the 6 biopsies from patients suffering from "possible" K.C.S. had normal lacrimal glands. Only one patient suffering from definite K.C.S. had a normal tissue biopsy. This patient was a 50 year old female with a two year history of sero positive rheumatoid arthritis, xerostomia and dry eyes. Her white blood count, serum globulin and liver function tests were normal, the erythrocyte sedimentation rate being slightly raised to 25 mm. in the first hour. Tests for antinuclear factor and salivary duct antibody were negative and x-ray and functional grades were zero. The patient's eyes had responded to artificial tear therapy (Chapter IX). All of the evidence, therefore, indicated that the patient had mild chronic rheumatoid arthritis and K.C.S. at the time the biopsy was carried out.

None of the remaining 23 patients with definite K.C.S. had lacrimal gland biopsies that showed entirely normal tissue (Table III,2). Mild chronic inflammation was detected in the one patient with primary biliary cirrhosis and K.C.S. This was a 67 year old female patient with a six year history of liver disease without arthropathy and a six months' history

of dry eyes. Salivary duct autoantibodies were present but there was no clinical, serological or x-ray evidence of rheumatoid arthritis. The patient's eyes had responded to artificial tear therapy. The remaining patient with mild chronic inflammation (Stage I) was a 75 year old female with osteoarthritis and xerostomia of six years' duration - sicca syndrome -. Eleven patients, 9 with Sjøgren's syndrome and two with the sicca syndrome had severe chronic inflammatory changes (Stage III) and the late results of inflammation (Stage III) were recorded in the remaining 9 patients, 8 with Sjøgren's syndrome and one sicca syndrome (Table III,2). No histological features distinguished the lacrimal glands of patients with Sjøgren's syndrome from those with K.C.S. and no rheumatoid arthritis.

Severity of lacrimal gland destruction tended to be greater with increased duration of ocular disease (Table III,3). Thus none of the 8 patients who had suffered from K.C.S. for less than three years had Stage III changes. On the other hand, three patients with a three to five year history showed late changes (Stage III) whereas four patients with a five to seven year history showed only mild or severe chronic infiltration (Stages I and II). The groups are too small to compare with respect to severity of arthritis. However, it is interesting to note that Sjøgren's syndrome is more likely to develop in severe rheumatoid arthritis of long duration (Chapter V) and more severe destruction of lacrimal gland might be expected to

correlate with advanced forms of arthritis.

Five of the Sjøgren patients had received systemic steroid therapy for periods varying from 2 to 10 years in an average daily dose of 5 mgm. of prednisolone or its equivalent per day. Stage III (late) changes were described in four and Stage II (Severe chronic) in one case. Steroid therapy had not prevented marked involvement of the lacrimal glands in these patients. All five patients had suffered from K.C.S. for more than five years and had received systemic steroids during that time. Nevertheless, no difference in histology could be determined between these patients and the remaining group who had never received systemic steroids and who had also suffered from K.C.S. for more than five years.

Discussion

Biopsy of the orbital portion of the lacrimal gland in patients suffering from K.C.S. had not been recorded in a series of patients until this study was conducted. From my perusal of the literature, it appears that no series of greater than 8 patients have been examined post mortem and in most instances only a single case has been reported (Sjøgren, 1933; and others listed in the introduction). Investigation of the recorded work further suggests that most specimens were obtained from extreme cases who had died as a

result of the complications of associated systemic disease and that lacrimal histology could be expected to show advanced changes. For these reasons, a series of 29 female patients depicting a range of involvement with K.C.S. were selected for lacrimal gland biopsy. The specimens could be classified into four stages; normal tissue, mild chronic, severe chronic inflammation, and a late fibrotic phase. In general, the severity of destruction paralleled the duration of ocular disease. The groups, however, were too small for definite conclusions regarding relationship with severity and duration of arthritis. Nevertheless, in a parallel clinical study reported in Chapter V, Sjøgren's syndrome was more prevalent in severe arthritis and severe lacrimal gland destruction might be expected to correlate with advanced arthritis. A future study of interest would be to extend the present series to include a wide range of arthritic patients who had suffered from K.C.S. for the same duration. Neither the 10 age matched patients examined post mortem, nor the 6 patients with "possible" K.C.S. had abnormal lacrimal gland tissue. This would suggest that the definition of Sjøgren's syndrome as a chronic inflammatory disease is valid. However, Radnot recorded over 30 years ago that patients over the age of 50 years undergo progressive atrophy of the lacrimal glands and that infiltration with lymphocytes is a normal occurrence in this and older age groups (Radnot, 1939). The mean age of the control group in this series was

64 years, somewhat older than the Sjøgren patients, 61.4 years, yet no abnormalities of the lacrimal glands were detected. However, another future study suggested is the extension of 'normal' lacrimal gland histology in older age groups. In this context, it is interesting to note that the prevalence of K.C.S. in hospital patients is less than in rheumatoid arthritis, except in geriatric patients of over 80 years of age (Chapter IV).

There were no specific features in the lacrimal gland that distinguished Sjøgren's syndrome from the sicca syndrome. Further comparison of these groups is studied in Chapter VI.

Systemic corticosteroid or adrenocorticotrophic hormone therapy may be effective in controlling acute exacerbations of Sjøgren's syndrome particularly when accompanied by swelling of the lacrimal and salivary glands (Manschot, 1961) but has little place in the treatment of the disease in its chronic form (Chapter IX). The latter view has been strengthened by the absence of any difference in lacrimal gland histology in steroid and non steroid treated patients who had experienced K.C.S. for similar periods.

Summary

Biopsies of the orbital portion of the lacrimal gland were carried out in 29 female patients, 18 Sjøgren's

syndrome, 4 sicca syndrome, one biliary cirrhosis and 6 with rheumatoid arthritis and "possible" keratoconjunctivitis sicca (K.C.S.). In addition 10 post mortem specimens were obtained from patients who had no history of ocular disease and who had died from diseases unrelated to rheumatoid arthritis or other connective tissue diseases. The biopsies were classified according to severity into normal, mild chronic inflammation, severe chronic inflammation and late fibrosis (Stages 0, I, II and III). All of the post mortem specimens and the 6 from patients with "possible" K.C.S. had normal lacrimal glands. The degree of infiltration with lymphocytes disruption of normal glandular tissue and eventual replacement with acellular fibrotic tissue was related to the duration of the K.C.S. There were no specific features in K.C.S. associated with rheumatoid arthritis (Sjøgren's syndrome) not seen in the sicca syndrome. Systemic steroid therapy did not appear to have influenced the microscopic features in the lacrimal glands of five patients who had suffered from K.C.S. for more than five years.

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CHAPTER IV

KERATOCONJUNCTIVITIS SICCA IN NON-RHEUMATIC SUBJECTS, INCLUDING THE ELDERLY

Sjøgren (1933) noted that keratoconjunctivitis sicca was recorded as a diagnosis in only 19 of 36,000 patients (0.05%) at the Sabbatsberg Hospital in Sweden, and Beetham (1935) could trace only one case in 20,000 attending the Massachusetts Eye and Ear Infirmary (0.0005%). However, de Roetth (1945) observed that one in 620 of his ophthalmic outpatients (0.16%) had keratoconjunctivitis sicca. Conversely, it took Gifford, Puntenney and Bellows (1943) four years to collect 33 keratoconjunctivitis sicca patients from a very busy practice and they suggested that more cases were being recognised each year. Furthermore, in 1950, Henderson reported that he was examining 13-14 new cases of Sjøgren's syndrome per year over a 9 year period. In none of these series however, was the age and sex distribution of all the patients described. Despite this, the influence of age and sex factors on the incidence of keratoconjunctivitis sicca, has been discussed by many authors. Earlier reports suggested the disease was exclusive to women at the menopause although by 1940, Sjøgren no longer considered the menopause was a significant factor.

There followed a series of publications citing examples of the disorder in patients younger than 40 years and in males (Bruce, 1941; Gifford and colleagues, 1943; Lutman and Favata, 1946; Stenstam, 1947; Holm, 1949; Henderson, 1950). Bloch, Buchanan, Wohl and Bunim in 1965 studied 39 patients with Sjøgren's syndrome and 23 with the sicca syndrome and found that

50 per cent of their female patients developed symptoms before the menopause. Although the commonest decades of onset were the fourth, fifth and sixth, there was no clear cut relationship with the menopause itself.

Reduction in tear secretion with age has been observed by some investigators (de Roetth, 1941; Henderson and Prough, 1950; Wright and Meger, 1962; Norn, 1965). Conversely, Holm (1949) concluded after examining the literature on histology of the lacrimal gland and a group of non-rheumatic patients, that there was no indication of reduced tear secretion with age.

Faced with such conflicting and incomplete surveys, I considered it of interest to study the prevalence of keratoconjunctivitis sicca in non-rheumatic male and female subjects of widely differing age groups. This study was all the more relevant since I had determined to examine the prevalence of Sjøgren's disease in organ specific autoimmune disorders and required a knowledge of the prevalence of keratoconjunctivitis sicca in the non-rheumatic patient for purposes of comparison.

Examination of a geriatric group of patients for keratoconjunctivitis sicca presents its own particular set of problems and interests. The prevalence of rheumatoid factors and antinuclear factors is high in Sjøgren's syndrome with and without rheumatoid arthritis (Bloch, Wohl, Ship, Oglesby and Bunim, 1960; Bunim, 1961; Crews and Whitfield, 1963; Bech, 1963; Bloch, Buchanan, Wohl and Bunim, 1965; Beck, Anderson, Bloch, Buchanan and Bunim, 1965) and also in the elderly patient

especially females (Laurence, 1961; Beck, 1963). It is therefore clearly of interest to examine the elderly patient for evidence of Sjøgren's syndrome in relation to rheumatoid and antinuclear factors. Mitochondrial antibodies are found in the serum of patients with primary biliary cirrhosis (Walker, Doniach, Roitt and Sherlock, 1965; Doniach, Roitt, Walker and Sherlock, 1966; Goudie, MacSween and Goldberg, 1966), cryptogenic cirrhosis or active chronic hepatitis (Doniach and colleagues, 1966) and occasionally in patients who give no clinical impression of liver disease; for example, low titre mitochondrial antibody has been described in Sjøgren's syndrome (Doniach and colleagues, 1966; Doniach and Walker, 1969). Conversely, clinical and/or biochemical evidence of liver disease was found in 6 per cent of patients with Sjøgren's syndrome without rheumatoid arthritis, 1.5 per cent of patients with Sjøgren's syndrome and rheumatoid arthritis and 0.66 per cent with rheumatoid arthritis alone (Whaley, Goudie, Williamson, Nuki, Dick and Buchanan, 1970). Clinical, biochemical and immunological evidence of liver disease was looked for in the geriatric group and correlated with Sjøgren's syndrome.

Material and Methods

Patients Studied

Four hospital sources were utilized.

Group 1 Orthoptic Patients (25)

These were children aged 5-15 years, mean 9.8, 12 girls and 13 boys, who were attending the Orthoptic Departments of the Ophthalmic Institute and Southern General Hospital for treatment of strabismus. None of the children had had acute rheumatism, Still's disease or congenital alacrima.

Group 2 Orthopaedic and Accident Outpatients (40)

These are young adults aged 16-30, mean age 21.3 years, 23 female and 17 male, who were attending the Accident and Orthopaedic Outpatient Department of the Royal Infirmary, as a result of a variety of arm and leg fractures and lacerations. None of the patients was known to be suffering from a systemic disease.

Group 3 Miscellaneous Medical Clinics (120)

These were adult patients aged 31-79, mean 56.4, 58 male and 72 female patients, attending clinics associated with the Western and Royal Infirmaries. They

had a variety of general medical conditions, none of which had any known association with rheumatic disease or autoimmune disorders.

Group 4 Geriatric patients (145)

These were inpatients in Cowglen and Shieldhall Hospitals and were all over the age of 80 years (57 males, 88 females, mean age 83.8 years). The variety of medical disorders for which they had been admitted to hospital is shown in Table IV,2. None of the patients was acutely ill or dehydrated.

The diagnosis of keratoconjunctivitis sicca was made in accordance with the criteria set out in Chapter II. All patients were examined for clinical evidence of xerostomia and sialograms were carried out in 36 of the patients in Group 3 (Park and Mason, 1966). Sialograms were not performed on the geriatric patients who were in the main too infirm to withstand the ambulance journeys and outpatient waiting entailed.

Laboratory Investigations

Laboratory investigations in the over 80 year old patients included serum alkaline phosphatase, total protein, serum albumin and globulin, total bilirubin and serum glutamic oxalacetic transaminase estimations carried

PREVALENCE OF KERATOCONJUNCTIVITIS SICCA (KCS)

in

NON-RHEUMATIC HOSPITAL PATIENTS

Clinical Groups	No. of patients	Age (yrs) Mean	Age (yrs) Range	Schirmer's test 11 of 5 minutes 5 No.	5 per cent	5 - 9 No.	5 - 9 per cent	10 - 14 No.	10 - 14 per cent	Keratoconjunctivitis sicca No.	Keratoconjunctivitis sicca per cent
Orthoptic patients	25	9.9	5 - 15								
Orthopaedic and Accident out-patients	40	21.3	16 - 30								
Miscellaneous Medical Clinics	120	56.4	31 - 79	7	6.2	3	2.5	1	0.6	8	6.5
Geriatric patients	145	83.8	80 +	40	27.6	21	15.1	32	22.0	23	16.0

Table IV,1

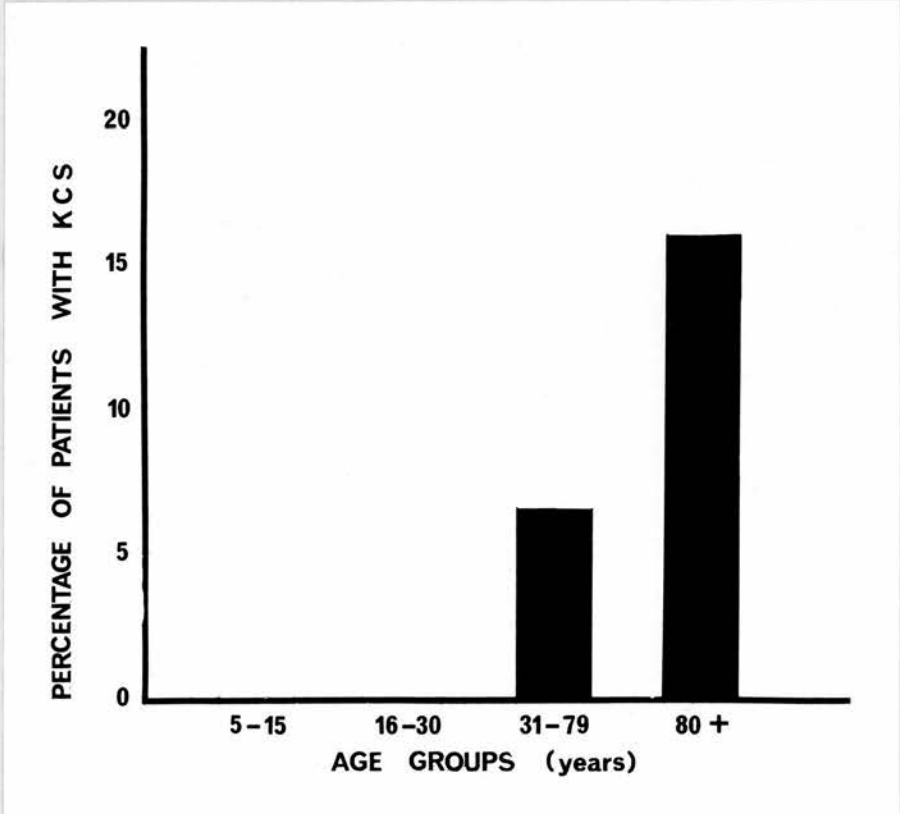

Fig.IV,1

out in accordance with the methods in current use in the Biochemistry Department of the Southern General Hospital, Glasgow. Additional data included antinuclear factors (by the indirect immunofluorescent technique) (Beck, 1961), latex fixation test (Singer and Plotz, 1966), sheep red cell agglutination test (Ziff, Brown, Lospallato, Baden and McEwan, 1956), mitochondrial antibodies to rat kidney (Goudie and others, 1966) and smooth muscle antibodies (Johnson, Holborow and Glynn, 1965).

Results

The results of the ophthalmological examinations are summarized in Table IV, 1 and in Figure IV,1. None of the patients in Groups 1 or 2 had keratoconjunctivitis sicca. A small number, 6.5 per cent in Group 3 had dry eyes. Two of the patients were under 50 years of age, the other six were between 51 and 65 years old. A significantly higher prevalence of keratoconjunctivitis sicca, 23 patients, 16 per cent, was detected in the Geriatric Hospital population ($P > 0.02$). The prevalence of dry eyes was no higher in the female group than the male (15 of 87 female, 8 of 58 male). Neither the four patients with pernicious anaemia nor the one with hypothyroidism had keratoconjunctivitis or xerostomia (Table IV,2).

No instances of xerostomia were observed in the

DIAGNOSIS IN PATIENTS OVER 80 YEARS OF AGE

Clinical Group	Number	Per cent
Cerebrovascular disease	101	69.6
Ischaemic heart disease	8	5.5
Orthopaedic casualties	9	6.2
Diabetes mellitus	5	3.4
Pernicious anaemia	5	3.4
Nutritional anaemia	5	3.4
Hypertension	4	2.7
Parkinson's disease	2	1.3
Rodent ulcer	1	0.6
Hypothyroidism	1	0.6
Paget's disease	1	0.6
Gangrene	1	0.6
Obesity	2	1.3
TOTAL	145	100.0

Table IV,2

LABORATORY DATA	Patients with keratoconjuncticitis sicca (23)	Patients without keratoconjunctivitis sicca (122)
Latex Fixation test	7 (30.6 per cent)	40 (33.3 per cent)
Anti-nuclear factor	8 (34.3 per cent)	35 (29.1 per cent)
Mitochondrical Antibodies	-	-
Smooth Muscle Antibodies	-	2 (1.6 per cent)
Total protein gm. per 100 ml	7.2 ± 2.1	7.3 ± 3.0
Serum Albumin gm. per 100 ml	4.9 ± 0.8	4.1 ± 0.75
Serum Globulin gm. per 100 ml	3.4 ± 0.8	3.6 ± 0.75
Serum Alkaline Phosphate (King Armstrong Unit) (mgm per 100 ml)	8.0 ± 3/100 ml.	8.4 ± 3.2/100 ml.
Total Bilirubin mgm/100 ml	0.48 ± 1.1	0.48 ± 1.0
Serum Glutamic Oxalacetic Transaminase. Units/ml.	28 ± 4	27 ± 4

Table IV,3 Laboratory data in the Geriatric Group of patients.

younger age groups. However, a dry mouth was noted in 7 of 36 (19% of patients) with miscellaneous medical disorders and abnormal sialograms were recorded in 6 of them (16%). Xerostomia was detected in 22 of the 145 (15% geriatric patients). Two female patients in Group 3 (1.6%) and 4 in the Geriatric Group (2.7%) had both xerostomia and keratoconjunctivitis sicca (the sicca syndrome). None of the patients had a history or clinical evidence of salivary or lacrimal gland enlargement. The results of the biochemical tests in the Geriatric Group of patients are shown in Table IV,3. There were no significant differences between those with keratoconjunctivitis sicca and those with normal tear secretion. A slightly raised γ globulin fraction was observed in 10 per cent of the sera, but this was not related to Sjøgren's syndrome in this group of patients.

The latex fixation test for rheumatoid factor was positive in 30-33 per cent of the geriatric patients although in weak titre in over two thirds of cases. In only 8 patients (17%) was the presence of rheumatoid factor confirmed by sheep red cell agglutination. Furthermore, of the 9 patients with strongly positive latex fixation test, 7 also had positive sheep red cell agglutination. None of the patients with both tests for rheumatoid factor however, had keratoconjunctivitis sicca or xerostomia.

Antinuclear factors were observed in 29-34 per cent of the geriatric sera, the prevalence being significantly higher in the female group (37 of 85 females, 6 of 58

male, $P > 0.02$). Fifteen patients had positive latex fixation tests and antinuclear factors and 5 of them were suffering from keratoconjunctivitis sicca - a finding which is of no statistical significance.

Although none of the geriatric patients had mitochondrial antibodies, four of them had antibodies to smooth muscle. None of these patients had keratoconjunctivitis sicca or xerostomia, clinical or biochemical evidence of liver disease.

Discussion

This study shows no evidence of keratoconjunctivitis sicca in patients aged 5 to 30. Only 6.5 per cent of non-rheumatic hospital patients aged 31 to 74 years had dry eyes; two were under 50 years and 6 between 50 and 65 years of age. However, the prevalence of keratoconjunctivitis sicca in the over 80 year old group of patients (16%) was significantly higher than in the younger age groups. The distribution of keratoconjunctivitis sicca between male and female elderly patients was equal. On the other hand, xerostomia occurred with equal frequency in groups 3 and 4. Furthermore, the co-existence of keratoconjunctivitis sicca and xerostomia (sicca syndrome) was observed in very few instances in both groups (1.6% and 2.7%).

The significance of autoantibodies in apparently non-diseased patients is not always immediately apparent,

e.g. it had been shown that euthyroid non-goitrous individuals may have thyroid autoantibodies, usually in low titre, and that these antibodies may increase with age, especially in females (Goudie, Anderson and Gray, 1959). Furthermore, thyroid autoantibodies may reflect a symptomatic focal Hashimoto's thyroiditis (Goudie and colleagues, 1959). In female patients these antibodies may be associated with abnormalities in iodine metabolism which are consistent with a mild Hashimoto's thyroiditis (Buchanan, Harden, Koutras and Gray, 1965). Gastric parietal cell autoantibodies also increase with age, especially females, and correlate with chronic asymptomatic atrophic gastritis (Irvine, 1956).

In contrast to these organ specific autoantibodies, the situation regarding non-organ specific autoantibodies such as rheumatoid and antinuclear factors is less clear. Certainly, rheumatoid factors (Laurence, 1961) and antinuclear factor (Beck, 1963) increase in prevalence with age especially in females, and are frequently found, albeit in usually low titre, in apparently healthy individuals. Indeed they may be found even in young persons although again in low titre (Buchanan, Boyle, Greig, McAndrew, Barr, Anderson and Goudie, 1966; Buchanan, Boyle, Greig, McAndrew, Barr, Gray, Anderson and Goudie, 1967). Since rheumatoid factor and antinuclear factor are frequently found in patients with Sjøgren's syndrome, even in the absence of rheumatoid arthritis or other connective tissue disease (Bloch and colleagues, 1965) and others quoted in the introduction, and since

keratoconjunctivitis sicca is found as is shown by this study in a proportion of healthy individuals, especially in the elderly, it might be thought that rheumatoid factor and antinuclear factor could be a reflection of keratoconjunctivitis sicca. However, in this geriatric series, this does not appear to be the case although one cannot exclude the possibility except by pathological studies, that is to say histological studies in the negative "keratoconjunctivitis sicca cases" with rheumatoid factor or antinuclear factor in their sera. It is possible that these patients could have histological subclinical keratoconjunctivitis sicca, and it is also possible that the diagnosis of keratoconjunctivitis sicca in the elderly, i.e. those over the age of 80, could be due to lacrimal gland failure sine inflammation.

Evidence of liver disease was sought in the geriatric group because patients with Sjøgren's syndrome exhibit evidence of liver disease (Whaley and colleagues, 1970) and patients with various liver diseases have mitochondrial antibodies as do patients with Sjøgren's syndrome (Walker and colleagues, 1965; Doniach and colleagues, 1966; Doniach and Walker, 1969). Clinical and biochemical evidence of liver disease was not present in any of the geriatric group although a slightly raised γ globulin fraction was observed in 10 per cent of cases. Four patients had antibodies to smooth muscle but no other abnormalities and no mitochondrial antibodies using rat kidney sections were detected. There appears, therefore, to be no evidence for an association between

Sjøgren's syndrome and a generalised active inflammatory process in the elderly patient.

Summary

Keratoconjunctivitis sicca is present in a number of "healthy" non-rheumatic patients. No evidence of the disease was found in 25 children or 40 young adults. A prevalence of 6.5 per cent of 120 outpatients whose mean age was 56.4 increased to 16 per cent of 145 geriatric patients whose mean age was 83.8. The difference is statistically significant. There was no indication in the geriatric group that the ocular disease was related to rheumatoid factors or antinuclear factors, to clinical, biochemical or immunological evidence of liver disease.

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CHAPTER V

KERATOCONJUNCTIVITIS SICCA IN RHEUMATOID ARTHRITIS

Although keratoconjunctivitis sicca (KCS) has been reported with varying prevalence (Chapter I, page 7) in rheumatoid arthritis, no systematized study of the relationship of K.C.S. to the various clinical and laboratory features of rheumatoid arthritis has been reported.

This chapter is a record of such a study in 893 adult patients suffering from classical or definite rheumatoid arthritis.

MATERIAL AND METHODS

Patients Studied

Eight hundred and ninety-three consecutive patients with definite or classical rheumatoid arthritis as defined by the diagnostic criteria of the American Rheumatism Association (Ropes, Bennett, Cobb, Jacox and Jessar, 1958) formed the basis of the study.

Each patient had a detailed ophthalmological examination as described in Chapter II. For the purposes of this study three groups were defined: patients with rheumatoid arthritis and no evidence of K.C.S., patients with rheumatoid arthritis and doubtful K.C.S., Patients with rheumatoid arthritis and definite K.C.S. Doubtful or possible K.C.S. was diagnosed when on two or more occasions the Schirmer II test was below 15 mm. of wetting and at the most only scattered staining of the conjunctivae with bengal rose was detected.

In addition to age and sex, the following clinical features were recorded in each patient: age of onset of arthritis, duration of arthritis, history of drug allergy, xerostomia and salivary gland enlargement, superficial and deep venous thrombosis, Raynaud's phenomenon, thyroid disease and skin ulcers.

On physical examination the following features were recorded: subcutaneous nodules, lymphadenopathy, splenomegaly, peripheral neuropathy, functional grade

and a clinical index of joint tenderness (Ritchie, Boyle, McInnes, Jasani, Dalakos, Grieveson and Buchanan, 1968).

X-ray joint surveys were interpreted according to the stages of Steinbrocker, Traeger and Batterman (1949).

Laboratory Investigations

Each patient had a full blood count including: haemoglobin estimation, total and differential white cell count, platelet count and erythrocyte sedimentation rate (Westergren). The serum iron, iron binding capacity, per cent saturation and Coombs test were also performed.

Biochemical tests included: total serum protein, serum albumin (Roberts, 1967), serum globulin (Storiko, 1968), thymol turbidity estimations.

Immunological tests included: rheumatoid factor by the sheep cell agglutination method (Ziff, Brown, Lospallato, Baden and McEwan, 1956), antinuclear factor (Beck, 1961), lupus erythematosus cell (Zinkham and Conley, 1956) and lupus erythematosus Latex tests (Dubois, Drexler and Arterberry, 1961), thyroglobulin autoantibody by precipitin (Anderson, Buchanan, Goudie and Gray, 1962) and tanned red cell haemagglutination (Fulthorpe, Roitt, Doniach and Couchman, 1961), thyroid antimicrosomal (Holborrow, Brown, Roitt and Doniach, 1959), gastric parietal cell

autoantibody (Adams, Glen, Kennedy, Mackenzie, Morrow, Anderson, Gray and Middleton, 1964), indirect immunofluorescence tests for salivary duct autoantibody (MacSween, Goudie, Anderson, Armstrong, Murray, Mason, Jasani, Boyle, Buchanan and Williamson, 1967), non-tissue specific precipitins (Anderson, Gray, Beck and Kinnear, 1961) and Venereal Diseases Reference Laboratory tests. Details of the immunological tests and the significance of salivary duct autoantibodies are recorded in Chapter VI.

The results of the examinations were recorded by the author on computer punch cards and subsequently transcribed on to magnetic computer tape. In order to go more deeply into the study of K.C.S. and its relationship with the clinical and laboratory features of rheumatoid arthritis, a multivariate analysis of all the data collected was carried out by calculating the discriminant function between the group of patients with R.A. and K.C.S. and those with R.A. and no evidence of K.C.S. (Kendall, 1961). Discriminant function was performed by the method described by Goulden (1952). Since this technique requires that all data on each variable be present, any case with missing observations was discarded. The occurrence of missing data in the patients' records was quite random, so omission of incomplete records does not appear to introduce any bias. Since many of the variables are inter-correlated the impact of all variables taken together is difficult to assess.

TABLE V,1

	R.A.			R.A. with doubtful K.C.S.			R.A. with definite K.C.S.		
	No.	Mean	St.Dev.	No.	Mean	St.Dev.	No.	Mean	St.Dev.
Age	759	50.77	12.921	60	54.75	10.549	74	57.81	9.297
Sex	759	1.72	0.452	60	1.73	0.446	74	1.80	0.405
Age Onset	759	42.71	13.493	60	46.01	12.926	74	42.77	11.970
Duration	759	8.05	7.870	60	8.74	10.597	74	15.04	12.788
Drug Allergy	757	0.12	0.330	60	0.12	0.324	73	0.15	0.360
Xerostomia	758	0.25	0.446	60	0.28	0.524	74	0.96	0.867
Salivary Gland Enlargement	758	$9.23^{\text{D}}-03$	0.096	60	0.02	0.129	74	0.08	0.275
Thrombosis	759	0.09	0.368	59	0.17	0.497	73	0.07	0.304
Raynaud's Phenomenon	759	0.29	0.606	60	0.25	0.541	74	0.61	0.841
Thyroid Disease	754	0.16	0.698	60	0.32	1.017	73	0.23	0.825
Skin Ulcers	750	0.02	0.131	59	0.03	0.183	73	0.01	0.117
Nodules	758	0.23	0.422	60	0.27	0.446	74	0.38	0.488

Table V,1

TABLE V,2

	R.A.			R.A. with doubtful K.C.S.			R.A. with definite K.C.S.		
	No.	Mean	St.Dev.	No.	Mean	St.Dev.	No.	Mean	St.Dev.
Lymphadenopathy	758	0.12	0.328	60	0.15	0.360	74	0.16	0.371
Splenomegaly	757	7.93@-03	0.089	60	0.00	0.000	74	0.00	0.000
Neuropathy	758	7.92@-03	0.089	60	0.00	0.000	74	0.00	0.000
Haemoglobin	744	12.61	2.017	60	12.39	1.711	74	12.30	2.382
E.S.R.	719	47.61	30.650	58	53.60	29.770	73	49.96	27.902
W.B.C.	716	7877.30	2591.701	59	7942.37	2554.405	71	7333.10	2917.251
Polymorphs	132	5297.03	2502.289	15	6460.60	2488.799	18	4590.56	1654.408
Lymphocytes	142	2069.01	1017.445	19	1790.05	683.602	20	2106.95	968.126
Platelets	424	2.74@+05	110767.127	39	2.67@+05	101848.390	51	2.93@+05	136429.463
Coombs	410	4.34	21.839	33	5.45	27.961	29	5.52	12.980
Serum Iron	80	43.13	37.370	4	19.50	7.594	15	39.00	31.657
Serum Iron Binding Capacity	79	279.90	86.137	4	262.50	35.707	15	276.20	87.735

Table V,2

TABLE V,3

	R.A.			R.A. with doubtful K.C.S.			R.A. with definite K.C.S.		
	No.	Mean	St.Dev.	No.	Mean	St.Dev.	No.	Mean	St.Dev.
Saturation %	78	15.22	11.107	4	7.50	3.697	15	13.07	11.164
Thymol Turbidity	700	2.53	1.633	57	2.65	1.885	70	2.76	2.442
Total Protein	737	6.99	0.593	59	6.85	0.603	73	6.94	0.675
Serum Albumin	733	3.41	0.495	59	3.15	0.498	73	3.24	0.427
Serum Globulin	733	3.57	0.722	59	3.70	0.625	73	3.69	0.673
V.D.R.L.	30	0.03	0.183	7	0.14	0.378	6	0.00	0.000
Sheep Cell Agglutination Test	752	5.74	3.250	60	6.62	3.173	74	5.66	3.417
Antinuclear Factor	728	1.48	2.781	59	2.98	4.041	73	2.58	3.135
Lupus Erythematosus Cell	22	0.05	0.213	2	0.00	0.000	1	0.00	0.000
Lupus Erythematosus Latex	472	$8.47^{\ominus}$-03	0.092	49	0.02	0.143	59	0.00	0.000
Thyroid Precipitin Test	47	0.06	0.247	1	0.00	0.000	2	0.00	0.000
Tanned Red Cell	744	0.79	2.442	60	1.00	2.972	73	0.82	2.697
Thyroid Microsomes	741	0.25	0.435	60	0.38	0.490	73	0.23	0.426
Gastric Antibodies	743	0.17	0.373	60	0.20	0.403	73	0.23	0.426

Table V,3

TABLE V,4

DISCRIMINANT ANALYSIS BETWEEN R.A. AND R.A. WITH DEFINITE K.C.S.

Variable Name	Discriminant Coefficient	T-Test for Contribution to Discrimination 591 Deg. Freedom	P Value
Age	0.0000208880	0.1409557410	N.S.
Sex	-0.0010802190	-1.6030387118	N.S.
Age at Onset	-0.0000531796	-0.3594834274	N.S.
Duration Arthritis	-0.0002515652	-1.6453387218	N.S. but is slightly higher
Nodules	-0.0016802672	-2.2855379336	< 0.05
S.C.A.T. Titre	0.0001732904	1.3146371833	N.S.
X-ray Stage	0.0005665354	2.0956856233	< 0.05
Articular Index	0.0000203754	1.0341977065	N.S. but is slightly higher
Functional Grade	-0.0013227776	-3.2250930073	< 0.002

N.S. = not significant

Table V,4

TABLE V,5

Variable Name	Discriminant Coefficient	T-Test for Contribution to Discrimination 823 Deg. Freedom	P Value
Drug Allergy	0.0002316364	0.4739801470	N.S.
Xerostomia	-0.0032543173	-10,5196059303	< 0.001
Salivary Gland Enlargement	-0.0038977556	-2.9280074178	< 0.01
Raynaud's Phenomenon	-0.0006768900	-2.5313413081	< 0.02

N.S. = not significant

Table V,5

Therefore, discriminant analyses with several different sets of variables were performed to determine whether the two groups could be more clearly differentiated on the basis of optimally weighting the clinical variables which had been recorded. The advantage of using a discriminant score is that several clinical variables and their complex interrelations are reduced to a single, easily understood number.

Results

The calculations were carried out with an I.B.M. 7040 Computer by means of a programme prepared at the Department of Biometrics and Medical Statistics, Standford University, California. The results of the computer print out are shown in Tables V, 1 to 5.

Seventy-four of the 893 patients in this study (8.3%) had keratoconjunctivitis sicca. No significant difference in age, sex, or age of onset of arthritis could be determined in the three groups (Table V, 1 and 4). Although the duration of arthritis was about twice as long in patients with definite K.C.S. as in those with R.A. and no K.C.S. the difference is short of significant (Table V,4) because of the large standard deviation in duration of the disease (Table V,1). Xerostomia and a history of salivary gland enlargement not surprisingly, were more frequent in the Sjøgren patients than in the rheumatoid arthritic series

(Tables V, 1 and 5). The significance of salivary duct autoantibody is the subject of Chapter VI. Other clinical features of importance are Raynaud's phenomenon, rheumatoid nodules, x-ray stage and functional grade, all of which are more severe in patients with R.A. and K.C.S. (Tables V, 1,4 and 5). There was no difference in the results of the laboratory investigations carried out in the groups studied (Table V,2).

Separate examination of the group with "doubtful K.C.S." was not useful. There were no clinical or laboratory features to distinguish them from the R.A. patients with no K.C.S. It is interesting to note that biopsies of lacrimal gland from six patients in this group showed normal tissue (Chapter III). Nevertheless, because it is not certain to which group these patients may belong their results were not used in the final statistical analyses.

Discussion

Although there was no significant difference in the age of onset in the K.C.S. group with R.A. and those with no K.C.S., the results are inconclusive. There was a relative paucity of patients whose disease began at the extremes of the spectrum. That is to say, the number of patients whose arthritis began in the second and third decade or in the eighth and over was

small. It is difficult to determine at which point a diagnosis of Still's disease (Juvenile rheumatoid arthritis) should be replaced by one of adult rheumatoid arthritis. One may use 15 years as the definitive age. Children with Still's disease have a low incidence of rheumatoid factors (under 6 years the tests are consistently negative), the prevalence increasing until at 15 years it approaches adult levels (Bywaters, Carter and Scott, 1959; Toumbis, Franklin, McEwen and Kuttner, 1963; Sievers, Ahvonen, Ahok and Wager, 1963; Cassidy and Walkenburg, 1967). Very rarely do adults develop rheumatoid arthritis over the age of 65 years. Nevertheless, the two extremes should be examined. Although the standard deviation of age of onset and duration of arthritis are quite high in this study (Table V,1), the wide range does not encompass sufficient numbers at the two extremes.

Sjøgren's syndrome occurs predominantly in females. Nevertheless, in rheumatoid arthritis, K.C.S. appears to be equally distributed between the sexes (Tables V,1 and 4). There are, of course, many more females with K.C.S. and R.A., since rheumatoid arthritis is more frequent in women. The result of this investigation indicates that rheumatoid arthritis predisposes both sexes equally to the development of K.C.S. A parallel might be drawn with thyrotoxicosis where the disease is also more common in the female but where affected males and females are equally prone to developing thyroid antibodies (Buchanan, Alexander,

Koutras, Crooks, Macdonald, Richmond and Wayne, 1962).

A high incidence of drug allergy in patients suffering from florid Sjøgren's syndrome has been reported (Bunim, Buchanan, Wertlake, Sokoloff, Bloch, Beck and Alepa, 1964) and early symptoms of Sjøgren's syndrome may be attributed to hay fever or similar allergies (Bloch, Buchanan, Wohl and Bunim, 1965). Nevertheless, in this study there was no evidence of a higher prevalence of drug allergy in patients with K.C.S. and R.A. over those with R.A. and no K.C.S. (Table V,5).

As expected xerostomia and salivary gland enlargement correlates closely with K.C.S. and R.A. The significance of salivary duct antibody and the result of labial gland biopsies are examined in the following Chapter.

Raynaud's phenomenon appears to be a feature of Sjøgren's syndrome irrespective of whether R.A. is present or not (Bloch and colleagues, 1965). In this study, of 74 patients with K.C.S. and R.A., Raynaud's phenomenon was significantly more frequent than in the R.A. patients without K.C.S. (Table V,5).

The correlation between subcutaneous nodules, severity of x-ray stage, functional grade and K.C.S. is high in this series of 893 patients. It is surprising that the sheep cell agglutination titre for rheumatoid factor (S.C.A.T. titre - Table V,4) does not correlate with K.C.S. since its correlation with rheumatoid nodules, advanced x-ray stage and

functional incapacity is high (Kellgren and Ball, 1959). Thus patients with a high titre of rheumatoid factor have severe joint disease (Vaughan, 1959; Ziff, 1957) and a poor prognosis (Duthie, Brown, Truelove, Baragar and Lawrie, 1964). In addition a high incidence of rheumatoid factor is found in patients with subcutaneous nodules (Ball, 1952; Kellgren and Ball, 1959). The poor correlation between R.A. with K.C.S. and the S.C.A.T. titre in this study cannot be explained at present. The articular index (Ritchie and Colleagues, 1968) is not significantly related but is slightly higher in those patients with K.C.S. and R.A. This result is not so surprising since, although there is a general trend for those with high index scores to have poor functional grades, the correlation is not particularly good (Ritchie and Colleagues, 1968).

Summary

The results of this study show that K.C.S. is more likely to occur in rheumatoid arthritic patients who have severe joint disease as judged by their x-ray stage and functional incapacity and in those with rheumatoid nodules. There is also a tendency for K.C.S. to occur with increasing duration of R.A. and in those with active joint inflammation. However, in this study age, age of onset, sex and sheep cell agglutination titre do not appear to be important factors in relation to the

development of K.C.S.

K.C.S. in rheumatoid arthritis correlates highly with the oral manifestations of Sjøgren's syndrome and with Raynaud's phenomenon. It does not correlate with the presence of drug allergy.

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CHAPTER VI

ASSOCIATION OF SJØGREN'S SYNDROME AND SALIVARY DUCT AUTOANTIBODY

In Sjøgren's disease, even in the absence of rheumatoid arthritis or other connective tissue disease (i.e. the sicca syndrome), there is hypergammaglobulinaemia and a high incidence of abnormal immunological reactions, such as antinuclear factors, rheumatoid factors, precipitating antibodies to tissue constituents, autoimmune complement-fixation tests, and passive cutaneous anaphylaxis in guinea-pigs (Jones, 1958; Stoltze, Hanlon, Pease and Henderson, 1960; Anderson, Gray, Beck and Kinnear, 1961; Anderson, Gray, Beck, Buchanan and McElhinney, 1962; Bunim, Buchanan, Wertlake, Sokoloff, Bloch, Beck and Alepa, 1964; Beck, Anderson, Bloch, Buchanan and Bunim, 1965; Bloch, Buchanan, Wohl and Bunim, 1965). In addition to these non-organ specific reactions, the prevalence of low titre thyroid auto-antibodies is slightly higher than expected (Anderson, Goudie, Gray and Buchanan, 1961; Bloch and others, 1965) and gastric parietal cell auto-antibodies with chronic atrophic gastritis show a higher prevalence, at least in patients studied in Glasgow (Buchanan, Cox, Harden, Glen, Anderson and Gray, 1966). These serum factors indirectly favour the view that Sjøgren's disease may have an autoimmune basis.

Bertram and Halberg (1964) and Halberg, Bertram, Søborg, and Nerup (1965) reported the demonstration by immunofluorescence of antibody against salivary duct epithelium in eleven of nineteen patients with Sjøgren's disease, and they considered that the antigen might be organ specific, i.e. peculiar to salivary tissue.

The incidence of this salivary duct antibody (SDA) in groups of patients with the sicca syndrome (Sc), patients with Sjøgren's disease complicated by rheumatoid arthritis (Sj-RA), patients with rheumatoid arthritis alone (RA) and patients with various other arthritides is recorded in the first part of this chapter.

Waterhouse and Doniach (1966) demonstrated focal lymphocytic sialadenitis in 16 of 17 patients with rheumatoid arthritis examined at post mortem. The possibility, therefore, exists that any salivary duct auto-antibody found in uncomplicated rheumatoid arthritis may reflect a subclinical form of Sjøgren's syndrome. Since biopsy of the major salivary glands is not without risks, and since the labial mucosal glands are frequently involved in Sjøgren's syndrome (Bloch and colleagues, 1965; Chisholm and Mason, 1968) it is of interest to study the association between focal lymphocytic sialadenitis of the labial glands and salivary duct auto-antibody in the serum of patients with Sjøgren's syndrome, rheumatoid arthritis or other arthritides and connective tissue diseases. The results of this investigation are recorded in the second part of this chapter.

INCIDENCE OF SALIVARY DUCT ANTIBODY IN VARIOUS CONDITIONS

Diagnosis	No. of Patients Studied	Sex		Age (yrs)		With Salivary Duct Antibody	
		Male	Female	Mean	Range	No.	Per cent.
Sicca Syndrome	13	1	12	64	53–78	2	15
Sjøgren's Disease with Rheumatoid Arthritis	17	4	13	55	29–81	11	65
Rheumatoid Arthritis	129	54	75	48	6–73	34	26
Systemic Lupus Erythematosus	4	—	4	35	20–60	1	25
Psoriatic Arthritis	9	1	8	49	20–65	—	—
Reiter's Syndrome	9	9	—	35	19–45	2	22
Ankylosing Spondylitis	1	1	—	43	—	—	—
Gout	6	4	2	58	36–83	—	—
Osteo-arthritis	43	12	31	58	23–78	—	—

Table VI,1

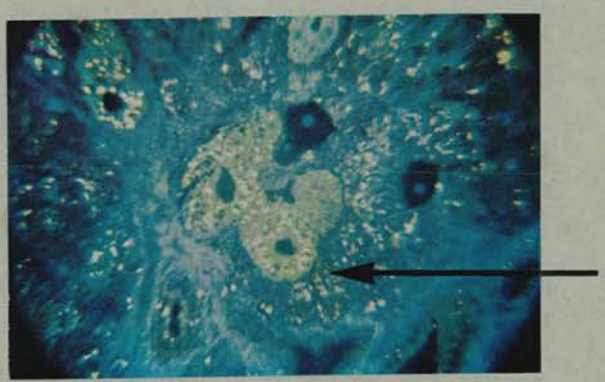


Fig. VI,1(a)

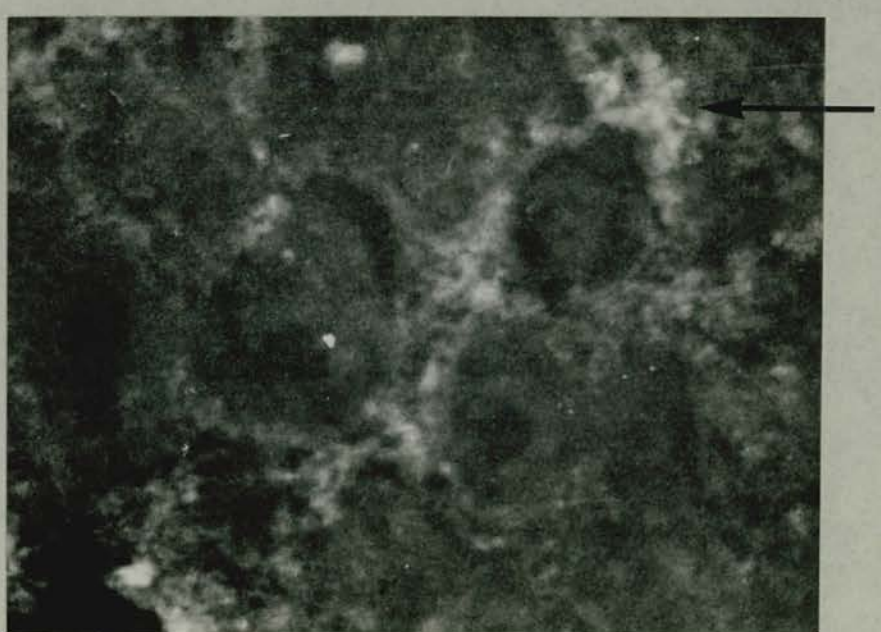


Fig. VI,1(b) Salivary duct autoantibody: the cytoplasm of salivary duct epithelial cells clearly fluoresces.

OCCURRENCE OF ANTIBODY TO SALIVARY DUCT EPITHELIUM IN SJØGREN'S DISEASE, RHEUMATOID ARTHRITIS AND OTHER ARTHRITIDES

Materials and Methods

Patients

231 patients were studied. The clinical diagnosis, sex distribution, mean age, and age range are shown in Table VI,1. The diagnosis of Sjøgren's disease was based on the criteria described in Chapter I and patients were required to show at least two of the three major components of the syndrome. The diagnosis of rheumatoid arthritis was based on the criteria of the American Rheumatism Association (Ropes, Bennett, Cobb, Jacox and Jessar, 1958).

Ophthalmological Examination

This was performed by the method described in Chapter II. For the purposes of this study a diagnosis of "definite" keratoconjunctivitis sicca was made when on two occasions the Schirmer II test showed wetting less than 15 mm. and the Rose Bengal dye test showed at least scattered staining of the conjunctivae and corneae. Patients with "possible" keratoconjunctivitis sicca had a Schirmer II of less than 15 mm. of wetting but no evidence of punctate or filamentary keratitis.

Each patient was carefully questioned regarding a history of xerostomia and of associated oral and pharyngeal symptoms of Sjøgren's disease (Bloch and others, 1965). Salivary flow studies were performed using a modified Carlson-Crittenden cup with an outer chamber diameter of 20 mm. and an inner chamber diameter of 10 mm. Parotid saliva was collected from each patient under resting condition and after stimulation with fruit gums and lemon juice.

Many patients admitted to having a dry mouth (symptomatic xerostomia) but without experiencing insufficiency of saliva and/or difficulty in mastication, or requiring increased fluid intake. Their mouths appeared to be moist and salivary flow studies on a sample of them were within the normal range.

Sialography was performed on all the 231 patients, using the hydrostatic technique described by Park and Mason (1966). The criteria of abnormality in the sialograms were based on those described by Bloch and others (1965).

Other Clinical and Laboratory Data

In addition to the age and sex of the patient and the ophthalmological and oral examinations described, the following clinical facts were recorded:

Duration of arthritis, presence of subcutaneous nodules, functional grade, and x-ray classification (Steinbrocker, Traeger and Batterman, 1949).

Laboratory investigations included:

Haemoglobin concentration, erythrocyte sedimentation rate (Westergren), white cell count, and assay of serum globulin.

Serological Methods

Salivary Duct Antibody (SDA) - Blocks of human submandibular gland obtained at autopsy not more than 10 hours after death were frozen on to metal chucks with CO_2 snow and 6μ sections were cut in a cryostat. The sera were applied undiluted to the unfixed section for 30 minutes at room temperature. After washing in normal saline buffered with veronal (pH 7.2) for 10 minutes, fluorescein-conjugated goat anti-human globulin serum was applied for 30 minutes. After a final 10 minutes wash in buffered saline the sections were mounted in buffered glycerol and examined with a Gillett and Sibert conference microscope using blue light. To reduce non-specific fluorescent staining, the fluorescein-conjugated anti-human globulin serum was absorbed twice with dried rat liver powder.

Antinuclear Factor (ANF) was detected using the indirect fluorescence method described by Beck (1961) with rat liver as substrate. The sera were initially tested at a dilution of 1 in 16 and positive sera were then titrated in quadrupling dilutions till an end point of nuclear staining was obtained.

Anti-thyroglobulin was detected by the tanned red cell haemagglutination test described by Fulthorpe, Roitt, Doniach and Couchman (1961), using thyroglobulin-coated formolized tanned sheep red cells (Burroughs Wellcome). The sera were initially tested at a dilution of 1 in 16 and positive sera were titrated in quadrupling dilutions.

Thyroid "Microsomal" Antibody was detected by the indirect immunofluorescence technique described by Holborow, Brown, Roitt and Doniach (1959), using unfixed thyrotoxic thyroid tissue as substrate and with the test sera diluted 1 in 4.

Gastric Parietal Cell Antibodies were demonstrated by an indirect immunofluorescence technique (Adams, Glen, Kennedy, Mackenzie, Morrow, Anderson, Gray and Middleton, 1964), using unfixed human gastric mucosa as substrate and testing the sera undiluted.

In the tests for SDA a highly reactive fluorescein-conjugated goat anti-human globulin provided by Dr. J.S. Beck was used, while in the other immunofluorescent tests commerically available fluorescein-conjugated rabbit anti-human globulin (Burroughs Wellcome) was used.

Rheumatoid Factor was determined by the Hyland latex (RA) test technique (Hyland Laboratories, (California). All sera were screened at a dilution of 1 in 32 and the presence of agglutination was recorded

15 and 45 seconds after mixing the reagents. Agglutination at either 15 or 45 seconds was recorded as positive. Positive sera were then titrated in doubling dilutions.

Non-specific Tissue Precipitin Tests were performed, using the method described by Anderson, Gray and others (1961) with human thyroid tissue as antigen. All specimens were tested undiluted and at a dilution of 1 in 8.

Results

Figure VI,1 shows positive and negative staining of salivary duct epithelium. Positive immunofluorescent staining varied in intensity, but even with the brightest staining pattern it was found that the antibody was present in low titre, none exceeding 1 in 32.

In the following statistical analysis X^2 has been calculated (when appropriate) using Yates's correction for small numbers. Comparisons which do not yield statistically significant differences are not discussed.

Whole Series: Incidence of SDA in Various Conditions (Table VI,1)

In patients with Sc, the antibody was found in only two of thirteen (15 per cent). In contrast, the

CLINICAL FINDINGS IN PATIENTS WITH SJØGREN'S DISEASE

Series			Sicca Syndrome (Sc)		Sjøgren's Syndrome and Rheumatoid Arthritis (Sj–RA)	
No. of Patients			13		17	
Salivary Duct Antibody			Present	Absent	Present	Absent
			2	11	11	6
Age (yrs)	Mean		64·0	64·6±8·3	56·8±11·3	55·8±16·6
	Range		62–66	53–78	33–63	29–55
Sex			2F	10F, 1M	8F 3M	5F, 1M
Keratoconjunctivitis Sicca	No. of Cases		2	11	11	5
	Duration (yrs)	Mean	4	7·9±6·7	8·7±8·4	14·5±8·6
		Range	2–6	1–21	<1–27	4–35
Xerostomia	No. of Cases		2	11	8	2
	Duration (yrs)	Mean	4	7·7±5·3	8·6±8·5	13·3±9·9
		Range	2–6	2–16	<1–25	5–31
Salivary Gland Enlargement			0	7	1	2
Abnormal Sialogram			2	10	3	2
Rheumatoid Arthritis	Duration (yrs)	Mean	—	—	8·5±8·7	15·2±11·1
		Range			5–27	6–35
	Functional Grade	I and II	—	—	9 (82%)	3 (50%)
		III and IV	—	—	2 (18%)	3 (50%)
	X ray Stage	I and II	—	—	6 (55%)	2 (33%)
		III and IV	—	—	5 (45%)	4 (66%)
	Subcutaneous Nodules		—	—	3	3

± Standard deviation

Table VI,2

LABORATORY FINDINGS IN PATIENTS WITH SJØGREN'S DISEASE

Series		Sicca Syndrome (Sc)		Sjøgren's Syndrome and Rheumatoid Arthritis (Sj–RA)	
No. of Patients		13		17	
Salivary Duct Antibody		Present	Absent	Present	Absent
		2	11	11	6
Haemoglobin (g. per cent.)	Mean	13·2	13·5±1·6	11·9±2·1	11·7±1·8
	Range	13·1–13·2	10·6–15·7	8·7–15·4	9·7–14·1
Erythrocyte sedimentation rate (Westergren)	Mean	48	27·3±16·9	55·1±33·2	66·5±45·1
	Range	36–60	2–52	6–108	8–119
White Cell Count (cells per c. mm.)	Mean	4,550	5,267±1,440	6,633±1,000	6,655±2,040
	Range	3,900–5,200	3,200–7,650	5,200–7,600	2,900–10,700
Serum Globulin (g. per cent.)	Mean	4·0	3·9±0·42	3·75±0·42	3·8±0·54
	Range	3·7–4·2	2·4–4·3	3·1–4·5	3·3–4·7
Rheumatoid Factor Positive		1	6	8	6
Antinuclear Factor Positive		1	3	2	6
Non-specific Tissue Precipitin Positive		0	4	2	1
Thyroglobulin Antibody		0	3	3	1
Thyroid Microsomal Antibody		1	5	3	2
Gastric Parietal Cell Antibody		0	4	5	1

± Standard deviation

Table VI,3

antibody was present in eleven of seventeen patients (65 per cent) with Sj-RA. In the RA group 34 of 129 patients (26 per cent) had SDA in their serum, an incidence not differing significantly from that found in the Sc group, but lower than that in the Sj-RA group ($X^2 = 12.23$; $P < 0.001$).

Of the various other groups examined, one of the four patients with systemic lupus erythematosus and two of nine males with Reiter's syndrome had SDA.

The patient with systemic lupus had definite keratoconjunctivitis sicca, severe xerostomia with objective evidence of reduced salivary flow rate, punctate sialectasis and intermediate duct changes on sialography, and a history of intermittent parotid swelling. Of the two patients with Reiter's syndrome, one had definite keratoconjunctivitis sicca, but no other stigmata of Sjøgren's disease were found.

None of the 43 patients with osteoarthritis was found to have SDA.

Sc and Sj-RA Groups (Tables VI,2 and 3)

Patients with Sj-RA had SDA more often than did those with Sc ($X^2 = 5.43$; $P < 0.02$). The two groups also differed in that the Sc patients were older ($t = 3.89$; $P < 0.001$), and had more sialographic abnormalities ($X^2 = 12.6$; $P < 0.001$) and a lower erythrocyte sedimentation rate ($t = 3.47$; $P < 0.001$). In Sj-RA there was a negative correlation between SDA and ANF ($X^2 = 8.24$; $P < 0.01$).

RELATIONSHIPS BETWEEN SALIVARY DUCT ANTIBODY AND CLINICAL FINDINGS IN 129 PATIENTS WITH RHEUMATOID ARTHRITIS

			Present	Absent	Significance
Salivary Duct Antibody			34	95	
Age (yrs)		Mean Range	53·3±12·2 25–79	44·4±15·8 6–71	$t=2·85$ $P<0·001$
Sex			16F, 18M	57F, 38M	—
"Possible" Keratoconjunctivitis Sicca			15 (44%)	20 (21%)	$\chi^2=6·67$ $P<0·01$
Symptomatic Xerostomia			14 (41%)	9 (9%)	$\chi^2=17·18$ $P<0·001$
Abnormal Sialogram			4 (12%)	3 (3%)	—
Rheumatoid Arthritis	Duration (yrs)	Mean Range	6·5±6·4 1/12–50	6·6±8·3 9/12–50	—
	Functional Grade	I and II III and IV	20 (59%) 14 (41%)	79 (83%) 16 (17%)	$\chi^2=8·31$ $P<0·01$
	x ray shape	I and II III and IV	7 (21%) 27 (79%)	47 (49%) 48 (51%)	$\chi^2=8·58$ $P<0·01$
	Subcutaneous Nodules		7 (21%)	12 (13%)	

± Standard deviation

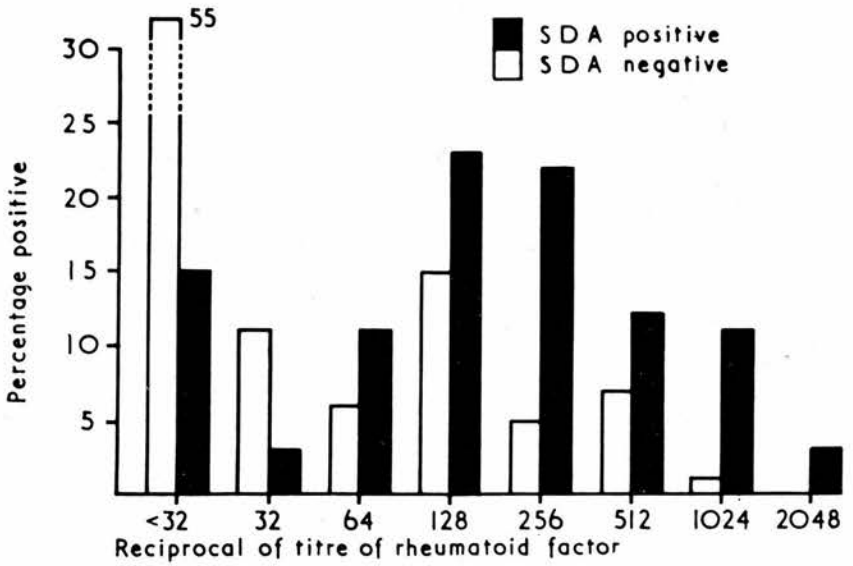
Table VI,4

RELATIONSHIPS BETWEEN SALIVARY DUCT ANTIBODY AND LABORATORY FINDINGS IN 129 PATIENTS WITH RHEUMATOID ARTHRITIS

		Present	Absent	Significance
Salivary Duct Antibody		34	95	
Haemoglobin (g. per cent.)	Mean Range	12·9±1·6 8·1–16·2	12·8±1·9 8·6–17·0	—
Erythrocyte sedimentation rate (Westergren)	Mean Range	50±30·1 5–114	33±27·2 2–125	$t=2·94$ $P<0·001$
White Cell Count (cells per c. mm.)	Mean Range	7,800±2,640 3,100–15,300	7,570±2,300 2,900–17,700	—
Serum Globulin (g. per cent.)	Mean Range	3·9±1·2 2·4–4·6	3·37±0·6 1·9–4·9	—
Rheumatoid Factor Positive		29 (85·3%)	43 (45·2%)	$\chi^2=16·27$ $P<0·001$
Anti-nuclear Factor Positive		13 (38%)	20 (21%)	—
Non-specific Tissue Precipitins Positive		1 (3%)	4 (4%)	—
Thyroglobulin		3 (9%)	7 (7%)	—
Thyroid Microsomal Autoantibody		8 (23%)	17 (18%)	—
Gastric Parietal Cell Antibody		9 (26%)	11 (11%)	—

± Standard deviation

Table VI,5



Histogram showing percentage of cases positive for rheumatoid factor in patients with rheumatoid arthritis with or without salivary duct antibody

Fig. VI,2

"POSSIBLE" KERATOCONJUNCTIVITIS SICCA, SYMPTOMATIC XEROSTOMIA, AND ABNORMAL SIALOGRAMS IN RHEUMATOID ARTHRITIS AND IN OTHER ARTHRITIDES

Diagnosis	Rheumatoid Arthritis			Other Arthritides*
	Total	Salivary Duct Antibody		
		Present	Absent	
No. of Patients	129	34	95	67
"Possible" Kerato-Conjunctivitis Sicca	35 (27%)	15 (44%)	20 (21%)	20 (30%)
Symptomatic Xerostomia	23 (27%)	14 (41%)	9 (9%)	12 (18%)
Abnormal Sialogram	7 (5%)	4 (12%)	3 (3%)	4 (6%)

*Other arthritides = psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, gout, and osteo-arthritis.

Table VI,6

RA Group (Tables VI,4 and 5; Fig. VI,2)

SDA was found significantly more frequently in older rheumatoid patients and in those with more severe rheumatoid disease as judged by functional grade, x-ray stage, erythrocyte sedimentation rate, and the prevalence of rheumatoid factor. As shown in Fig. VI,2, the prevalence of rheumatoid factor for all titres except 1 in 32 as well as the highest titres were seen in SDA positive patients.

A higher incidence of "possible" keratoconjunctivitis sicca and of symptomatic xerostomia was found in those with SDA. Table VI,6 compares the frequency of "possible" keratoconjunctivitis sicca, symptomatic xerostomia, and abnormal sialograms in rheumatoid patients and in those with other rheumatic diseases - psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, gout, and osteoarthritis. There is no significant difference. The sub-group of rheumatoid patients with SDA had symptomatic xerostomia more frequently than other patients ($X^2 = 6.4$; $P < 0.01$).

Comparison of Sj-RA and RA Groups

Among all the patients with rheumatoid arthritis, Sj-RA was found in those who were older ($t = 2.1$; $P < 0.05$), had had their arthritis for a longer period ($t = 1.98$; $P < 0.05$), and had more severe rheumatoid disease as judged by the erythrocyte sedimentation rate

($t = 3.5$; $P < 0.02$) and the presence of subcutaneous nodules ($X^2 = 4.5$; $P < 0.05$). The Sj-RA group also had a higher prevalence of SDA ($X^2 = 12.23$; $P < 0.001$) and of rheumatoid factor ($X^2 = 4.37$; $P < 0.05$).

Similarly, when Sj-RA patients were compared with SDA-positive RA patients, the former were shown to have had their arthritis longer ($t = 1.73$; $P < 0.05$) and to have a higher erythrocyte sedimentation rate ($t = 2.4$; $P < 0.02$). No age difference was, however, found.

Discussion

Bertram and Halberg (1964) first described the occurrence in Sjøgren's disease of an antibody against salivary duct epithelium. Since sera containing the antibody did not give immunofluorescent staining of salivary gland acini or of thyroid, they considered that the antibody might be specific for an antigen peculiar to salivary duct epithelium. Feltkamp (1967) has shown that the antibody could be absorbed from the serum with extracts of salivary gland, but extracts of a number of other tissues, including thyroid, liver, and kidney, failed to do so.

This study shows that the antibody reacts with the individual's own tissues (i.e. it is an auto-antibody) and also causes immunofluorescent staining of small lacrimal ducts, but not of gastric, thyroid, or prostatic epithelium. The mitochondral antibody found

in a high percentage of patients with primary biliary cirrhosis (Walker, Doniach, Roitt and Sherlock, 1965; Goudie, MacSween and Goldberg, 1966) gives an immunofluorescent staining pattern with salivary gland similar to that seen with SDA positive sera. Preliminary experiments, however, have shown that the SDA differs from the mitochondrial antibody in that only the latter can be absorbed from sera with rat liver mitochondria. The SDA thus shows some organ-specificity, but final confirmation must await further experimental investigation.

The present studies have found SDA in 15 per cent of patients with Sc, but in 65 per cent of patients with Sj-RA. In RA the incidence of the antibody was 26 per cent. In none of the large series of patients with osteoarthritis was the antibody present. The antibody is thus not peculiar to Sjøgren's disease. It is most commonly found in Sj-RA, and in one in seven of Sc patients. These observations suggest that the antibody is in some way related to the rheumatoid disease process, whether or not there be clinical evidence of salivary gland involvement. This is further emphasized in that, among RA patients, SDA was found significantly more frequently in older patients and those with more severe rheumatoid disease. Furthermore, Sj-RA also occurred in patients who were older, had had their arthritis longer, and had more severe rheumatoid disease. Circumstantial evidence of lacrimal and salivary gland involvement by the rheumatoid process was provided by

COMPARISON OF SICCA SYNDROME (Sc) WITH SJØGREN'S DISEASE WITH RHEUMATOID ARTHRITIS (Sj-RA)
Bloch and others (1965) and Bunim and others (1964)

Diagnosis		Sc	Sj-RA	Reference
Serum Globulin (g./100 ml.)	Mean Range	4·4 2·8–6·8	3·6 2·0–5·7	Bloch and others (1965)
Antinuclear Factor		14/16 (88%)	14/25 (56%)	Bloch and others (1965)
Pattern of Nuclear Fluorescence Staining	Homogeneous	7/16	8/18	Bloch and others (1965)
	Speckled	5/16	2/18	
	Nucleolar	5/16	0/18	
Auto-immune Complement-Fixation Test		15/19	5/26	Bloch and others (1965)
Precipitating Autoantibodies		13/16	1/18	Bloch and others (1965)
Anti-Gm Factors		4/20	14/27	Bunim and others (1964)
Reticulum Cell Sarcoma		4/23	0/32	Bloch and others (1965)

Numerator = number of patients with positive tests
Denominator = number of patients tested

Table VI,7 Resume of previous studies.

the significantly higher incidence of "possible" keratoconjunctivitis sicca and of symptomatic xerostomia noted in the SDA positive RA patients as compared with the RA patients without the antibody. This might suggest that a subclinical form of Sjøgren's disease was present in the SDA positive RA patients.

Histological evidence of salivary gland involvement in rheumatoid arthritis was provided by Waterhouse and Doniach (1966), who found focal lymphocytic sialadenitis in all of twelve females and in four of five males with rheumatoid arthritis. They considered the salivary lesion regularly found in rheumatoid arthritis to be Sjøgren's disease in miniature. It is thus perhaps not entirely surprising that, in rheumatoid arthritis, without clinical evidence of salivary or lacrimal gland involvement, there should be a high incidence of salivary duct antibodies.

The finding of a significantly lower incidence of SDA in the Sc patients than in the Sj-RA patients is of considerable interest. The previous detailed studies of Bloch and others (1965), Beck and others (1965), and Bunim and others (1964) - summarized in Table VI,7 - have shown differences between these two sub-groups of Sjøgren's disease. Furthermore, Talal, Leventhal and Waldorf (1966) found that lymphocytic transformation in response to phytohaemagglutinin and streptolysin was less in Sj-RA than in Sc. However, with dinitrochlorobenzene skin sensitization, these workers found that differences between the two groups were not apparent. Reference to

Table VI,7 shows that, with the exception of anti-Gm factors, non-organ-specific autoantibodies have been found to be consistently more prevalent in the Sc patients. It is therefore surprising that in this study in patients with Sc, which clinically appears to be an organ-specific disease, the possibly organ-specific SDA should be significantly less common than in the Sj-RA patients. The number of Sc patients is small in this series, but the findings, taken in conjunction with the observations of other workers, clearly indicate the need for a more detailed comparison of Sc patients and patients with salivary and lacrimal gland involvement accompanied by a connective tissue disorder.

Summary

(1) An immunofluorescent autoantibody to salivary duct epithelium has been found in two of thirteen patients with sicca syndrome, in eleven of seventeen patients with Sjøgren's disease and rheumatoid arthritis, and in 34 of 129 patients with uncomplicated rheumatoid arthritis.

(2) In patients with rheumatoid arthritis, the antibody was found significantly more frequently in older patients and in those with more severe rheumatoid disease.

(3) The antibody appears to be a manifestation of the rheumatoid disease process, in which other workers have shown a high incidence of chronic focal sialadenitis.

Salivary duct autoantibody is found in approximately 15% of patients with the sicca syndrome (keratoconjunctivitis sicca, xerostomia with or without salivary gland enlargement, but not rheumatoid arthritis or other connective tissue disease), 65% of patients with rheumatoid arthritis and Sjøgren's syndrome, and in 26% of patients with rheumatoid arthritis alone (vide supra). Feltkamp and Van Rossum (1968), however, found the salivary duct autoantibody in the sera of approximately 50% of patients with the sicca syndrome and also in the same number of patients with Sjøgren's syndrome with rheumatoid arthritis. The reason why patients with rheumatoid arthritis who have Sjøgren's syndrome develop this autoantibody is unknown. However, Waterhouse and Doniach (1966) demonstrated focal lymphocytic sialadenitis in sixteen of seventeen patients with rheumatoid arthritis examined at post-mortem. The possibility, therefore, exists that the salivary duct autoantibody found in uncomplicated rheumatoid arthritis may reflect a subclinical form of Sjøgren's syndrome (vide supra).

Since biopsy of the major salivary glands is not without risks, and since the labial mucosal glands are frequently involved in Sjøgren's syndrome (Bloch et al, 1965; Chisholm and Mason, 1968), it was thought to be of interest to study the association between focal lymphocytic sialadenitis of the labial glands and salivary duct autoantibody in the serum of patients with Sjogren's syndrome, rheumatoid arthritis or other arthritides and connective tissue diseases.

Labial mucosal lymphocytic sialadenitis and salivary duct antibody in the clinical groups studied

Diagnosis	No. of patients studied	Sex		Age (years)		With lymphocytic sialadenitis		With salivary duct antibody	
		Male	Female	Mean	Range	No.	%	No.	%
Sicca syndrome	10	2	8	55·9	27–66	6	60·0	1	10·0
Rheumatoid arthritis and Sjøgren's syndrome	27	6	21	60·3	48–78	17	63·0	19	70·4
Rheumatoid arthritis alone	47	13	34	53·2	20–73	13	27·7	21	44·6
Psoriatic arthritis	11	4	7	49·3	19–88	2	18·2	—	—
Ankylosing spondylitis	10	5	5	47·8	23–73	2	20·0	1	10·0
Reiter's syndrome	6	6	—	34·5	18–52	—	—	—	—
Still's disease	1	—	1	29·0	—	—	—	—	—
Systemic lupus erythematosus	1	—	1	21·0	—	—	—	—	—
Progressive systemic sclerosis	4	—	4	32·0	16–40	1	20·0	—	—
Gout	2	1	1	61·0	60–62	—	—	—	—
Osteoarthritis	11	3	8	64·9	53–74	1	9·1	—	—

Table VI,8 Occurrence of labial mucosa sialadenitis and salivary duct antibody in various clinical groups.

SALIVARY DUCT AUTOANTIBODY IN SJØGREN'S SYNDROME AND FOCAL SIALADENITIS IN THE LABIAL MUCOSA

Material and Methods

One hundred and thirty patients were included in the study. The clinical diagnosis, age and sex distribution are shown in Table VI,8.

Labial Mucous Membrane Biopsy

The biopsy was taken from the lower lip under local anaesthesia (Chisholm and Mason, 1968).

Results

The histological appearances of the buccal mucous membrane were divided into three types, (a) normal; Fig.VI,3; (b) diffuse lymphocytic infiltration; Fig.VI,4, and (c) a focal lymphocytic sialadenitis; Fig.VI,5. Both diffuse and focal infiltrations could be further subdivided into two stages, based on the severity of the infiltrate (Chisholm and Mason, 1968). In this way five stages of lymphocytic infiltration in the buccal mucous membrane biopsy were identified.

Stage 0 - Normal biopsy appearance.

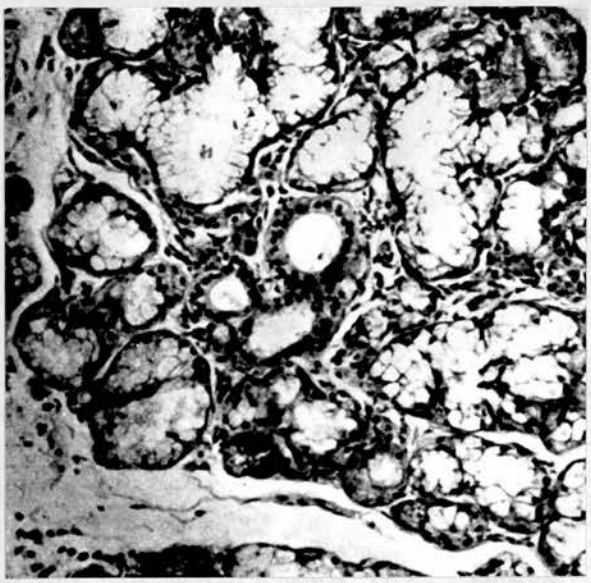


Fig.VI,3 Normal buccal membrane.

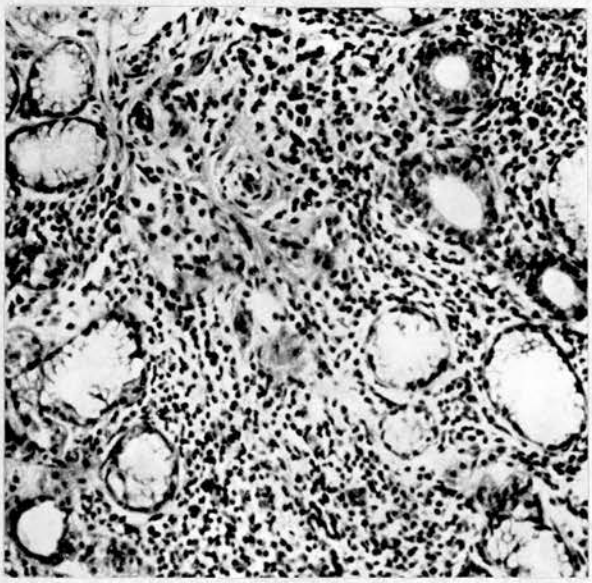


Fig.VI,4 Diffuse lymphocytic infiltration.

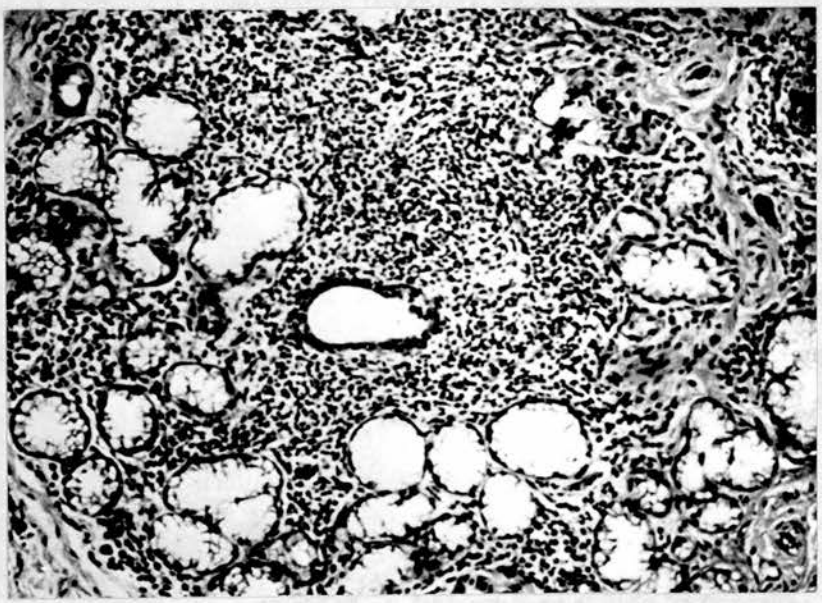


Fig.VI,5 Focal lymphocytic sialadenitis.

Stage I - Fine diffuse lymphocytic infiltrate.

Stage II - Marked diffuse lymphocytic infiltrate.

Stage III - Focal infiltration of lymphocyte.

Stage IV - Very heavy focal infiltration of lymphocytes.

Both Stage III and IV, in addition to foci of lymphocytes showed a diffuse infiltration of lymphocytes. In post-mortem studies, stages I and II were quite common (Chisholm and Mason, 1968) and so these were not included as positive in this series.

Table VI,8 shows the prevalence of salivary duct autoantibody and focal lymphocytic sialadenitis in the clinical groupd studied.

Salivary duct autoantibody was present in 10% of patients with the sicca syndrome, in 70.4% of patients with Sjøgren's syndrome and rheumatoid arthritis, and 44.6% of patients with rheumatoid arthritis uncomplicated by Sjøgren's syndrome. The prevalence of salivary duct autoantibody in patients with the sicca syndrome and Sjøgren's syndrome and rheumatoid arthritis is similar to that found in the study reported in part one of this chapter but the prevalence of salivary duct autoantibody in patients with rheumatoid arthritis alone is higher (thirty-four of 129 patients, 26%).

Of the forty-six patients with other arthritides and connective tissue diseases only one had salivary duct autoantibody present. This was a 63-year old woman with definite ankylosing spondylitis and a positive sheep cell agglutination test for rheumatoid factor at a titre of 1:256, but without clinical evidence of Sjøgren's syndrome or rheumatoid arthritis.

The prevalence of labial focal lymphocytic sialadenitis was the same in patients with sicca syndrome and Sjøgren's syndrome and rheumatoid arthritis (60 and 63%, respectively). The prevalence in rheumatoid arthritis alone was 27.7%.

Two patients with psoriatic arthropathy had focal lymphocytic sialadenitis, but neither had salivary duct autoantibody. One of these patients had 'possible' keratoconjunctivitis sicca.

Two patients with ankylosing spondylitis had focal lymphocytic sialadenitis, and one of these, mentioned above, had salivary duct autoantibody present. Neither of these two patients had any evidence of Sjøgren's syndrome.

Of the four patients with progressive systemic sclerosis one had focal lymphocytic sialadenitis, but no evidence of Sjøgren's syndrome.

One patient with osteoarthritis had a positive buccal mucosal biopsy and had evidence of 'possible' keratoconjunctivitis sicca. No salivary duct autoantibody was detected in this patient's serum.

The relationship of the salivary duct autoantibody

Laboratory and immunological features of patients with sicca syndrome

	Present	Absent	Significance
Focal lymphocytic sialadenitis	6	4	
Haemoglobin (g/100 ml)			
Mean ± SD	12·9	13·4	
Range	12·0–13·8	11·3–13·7	—
White cell count (per mm³)			
Mean ± SD	5,850	6,150	
Range	3,300–7,800	5,000–9,700	—
Erythrocyte sedimentation rate (mm/1st hr—Westergren)			
Mean ± SD	24	15	
Range	14–38	3–32	
Serum globulin (g/100 ml)			
Mean ± SD	3·3	3·5	
Range	2·1–4·2	2·7–4·1	—
Salivary duct antibody	1 (15%)	0	—
Rheumatoid factor	5 (83%)	3 (75%)	—
Antinuclear factor	2 (33%)	1 (25%)	—
Non-tissue specific precipitating autoantibody	—	—	—
Thyroglobulin autoantibody	1 (13%)	2 (50%)	—
Thyroid microsomal autoantibody	1 (17%)	1 (25%)	—
Gastric parietal cell autoantibody	—	—	—

Table VI,9

Laboratory and immunological features of patients with Sjøgren's syndrome and rheumatoid arthritis

	Present	Absent	Significance
Focal lymphocytic sialadenitis	17	10	
Haemoglobin (g/100 ml)			
Mean ± SD	12·2	12·9	
Range	11·9–15·4	5·0–16·1	—
White cell count (per mm³)			
Mean ± SD	6,946	6,297	
Range	3,400–14,600	1,260–10,500	—
Erythrocyte sedimentation rate (mm/1st hr—Westergren)			
Mean ± SD	42 ± 26·9	65 ± 42·9	
Range	12–121	8–125	—
Serum globulin (g/100 ml)			
Mean ± SD	3·6	3·8	
Range	1·8–4·5	2·9–5·2	—
Salivary duct antibody	12 (61%)	7 (70%)	—
Rheumatoid factor	16 (94%)	7 (70%)	—
Antinuclear factor	8 (47%)	3 (30%)	—
Non-tissue specific precipitating autoantibody	4 (23·5%)	—	—
Thyroglobulin autoantibody	3 (17·6%)	1 (10%)	—
Thyroid microsomal autoantibody	5 (29·4%)	3 (30%)	—
Gastric parietal cell autoantibody	4 (23·5%)	—	—

Table VI,10

Laboratory and immunological features of patients with rheumatoid arthritis

	Present	Absent	Significance
Focal lymphocytic sialadenitis	13	34	
Haemoglobin (g/100 ml)			
Mean ± SD	11·3 ± 1·7	13·0 ± 1·65	
Range	8·7–14·4	7·1–15·3	$P < 0.001$
White cell count (per mm³)			
Mean ± SD	6,108 ± 2,471	8,201 ± 3,023	$P < 0.02$
Range	2,000–11,600	5,000–15,900	
Erythrocyte sedimentation rate (mm/1st hr—Westergren)			
Mean ± SD	78 ± 19·3	41 ± 26·1	
Range	36–107	5–106	$P < 0.001$
Serum globulin (g/100 ml)			
Mean ± SD	4·0 ± 0·65	3·6 ± 0·68	
Range	3·2 –5·5	2·6 –5·2	—
Salivary duct antibody	5 (38·5%)	16 (47·1%)	—
Rheumatoid factor	13 (100%)	29 (85·3%)	—
Antinuclear factor	7 (53·8%)	6 (17·6%)	$P < 0.02$
Non-tissue specific precipitating autoantibody	3 (23·1%)	2 (5·9%)	—
Thyroglobulin autoantibody	5 (38·5%)	6 (17·6%)	—
Thyroid microsomal autoantibody	4 (30·8%)	7 (20·6%)	—
Gastric parietal cell autoantibody	2 (15·4%)	9 (26·5%)	

Table VI,11

and other autoantibodies and laboratory data in patients with the sicca syndrome, Sjøgren's syndrome and rheumatoid arthritis, and rheumatoid arthritis alone is shown in Tables VI,9-11. It can be seen that in none of these three groups did the salivary duct autoantibody correlate with focal lymphocytic sialadenitis. None of the other autoantibodies including rheumatoid and antinuclear factors, non-tissue specific precipitins, anti-thyroglobulin, anti-thyroid 'microsomes' and gastric parietal cell autoantibodies correlated with focal lymphocytic sialadenitis in any of the three clinical groups, with the exception of the antinuclear factor in patients with rheumatoid arthritis (Table VI,11) ($P < 0.02$). None of the other laboratory features correlated with the finding of focal lymphocytic sialadenitis in patients with Sjøgren's syndrome whether associated with rheumatoid arthritis or not. In patients with rheumatoid arthritis alone the haemoglobin ($P < 0.001$) and white cell count ($P < 0.02$) were significantly lower in patients with focal lymphocytic sialadenitis and the erythrocyte sedimentation rate was significantly higher ($P < 0.001$).

Discussion

Sjøgren's syndrome is a chronic benign disorder which is characterized by chronic inflammatory changes, not only in the lacrimal and major salivary glands, but

also in the small mucus-secreting glands of the conjunctiva, mouth, nasal passages, pharynx, trachea and bronchi and also in the sweat glands (Bloch et al, 1965). In this study biopsy of the labial mucous membrane has been used to investigate the relationship of salivary duct autoantibody to focal lymphocytic sialadenitis of the oral mucosa in patients with Sjøgren's syndrome with or without rheumatoid arthritis, rheumatoid arthritis alone, and other arthritides and connective tissue disorders.

Salivary duct autoantibody was present in only one of ten patients with the sicca syndrome (Sjøgren's syndrome uncomplicated by rheumatoid arthritis or other connective tissue disease), although six had focal lymphocytic sialadenitis on labial mucosal biopsy. In contrast, nineteen of the twenty-seven patients (70.4%) with rheumatoid arthritis and Sjøgren's syndrome had salivary duct autoantibody, although the prevalence of focal lymphocytic sialadenitis in this group was the same as in patients with the sicca syndrome. This suggests that salivary duct autoantibody may be a manifestation of Sjøgren's syndrome associated with rheumatoid arthritis rather than a reflection of Sjøgren's syndrome per se. This conclusion is supported by the finding of a very high prevalence of salivary duct autoantibody in patients with rheumatoid arthritis alone (twenty-one of forty-seven, 44.6%) and the comparatively low prevalence of focal lymphocytic sialadenitis in this group (thirteen of forty-seven,

27.7%). This high incidence of the salivary duct autoantibody probably represents a selection bias. Furthermore, there was a complete lack of correlation between salivary duct autoantibody and focal lymphocytic sialadenitis in patients with the sicca syndrome, Sjøgren's syndrome with rheumatoid arthritis, and rheumatoid arthritis alone. These findings are in agreement with those of Bertram (1967) who performed palatal biopsies on eight patients with Sjøgren's syndrome, six (75%) of whom had heavy lymphocytic and plasmacytic infiltrates and occasional myoepithelial cell islands. Of these six patients only two had salivary duct autoantibody present in their serum.

It may be argued that the lack of correlation between the salivary duct antibody and lymphocytic sialadenitis may have been due to a sampling error, the changes in the labial mucosa being patchy rather than generalized. However, in twelve post-mortem specimens, identical changes were found when biopsies were taken from both sides of the mouth. It is also possible that biopsy of the major salivary glands may have shown a correlation, but it is not feasible to carry out biopsy of these glands during life since the procedure carries definite risk of injury to the facial nerve, and of salivary fistulae, as well as leaving the patient with a scar. However, in post-mortem studies performed by Chisholm and Waterhouse (1968) a positive correlation between the findings in the submandibular and labial minor salivary glands could be made ($P < 0.01$).

The salivary duct antibody reacts with the epithelial cytoplasm of the lacrimal and salivary glands and has been shown to have autoreactivity in one case above (page 103) and in a further two cases in this series. It has been shown by Feltkamp and Van Rossum (1968) to be absorbed by extracts of salivary tissue but not by extracts of human pancreas, liver, thyroid, adrenal or muscle. The apparent organ-specificity of the antibody, therefore, corresponds to the occurrence of inflammatory lesions in lacrimal and salivary gland in Sjøgren's syndrome. Nevertheless, from the present findings, salivary duct antibody appears to be an epiphenomenon associated with the pathological changes of Sjøgren's syndrome complicating rheumatoid arthritis rather than with Sjøgren's syndrome occurring alone.

Summary

No correlation between the occurrence of the salivary duct antibody and focal lymphocytic sialadenitis in the labial mucosa was found in ten patients with the sicca syndrome, twenty-seven patients with Sjøgren's syndrome and rheumatoid arthritis, and forty-seven patients with rheumatoid arthritis alone. No correlation between the variables was found in any of the groups examined. Post-mortem studies on the labial mucosal biopsy show that the results of biopsy are reproducible, and so the lack of correlation is not due to sampling

error when the biopsy is taken. It is suggested that the salivary duct antibody is an epiphenomenon of rheumatoid arthritis rather than a manifestation of Sjøgren's syndrome per se.

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CHAPTER VII

ASSOCIATION OF SJØGREN'S SYNDROME AND AUTO-IMMUNE THYROID DISEASE, PERNICIOUS ANAEMIA AND IDIOPATHIC ADDISON'S DISEASE

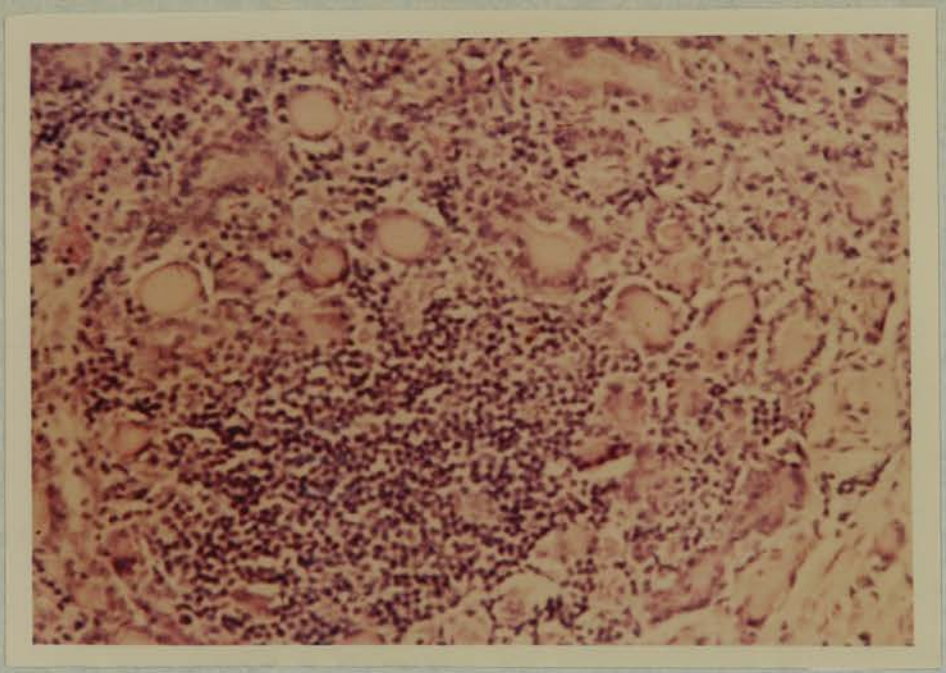

Fig. VII,1 X4 Magnification of section of thyroid gland in Hashimoto's thyroiditis

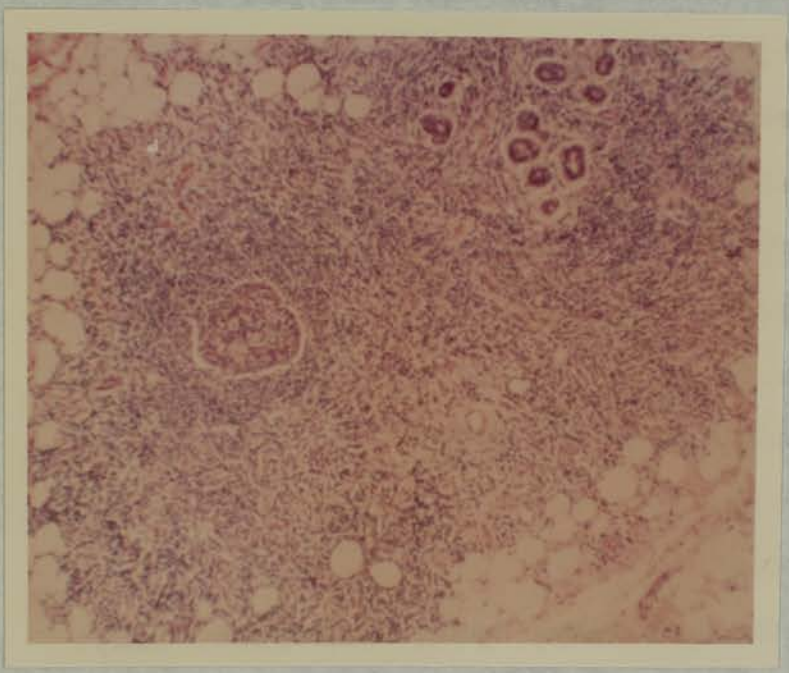

Fig. VII,2 X10 Magnification, Hashimoto's thyroiditis.

Compare with Figs.III, Chapter III lymphocytic infiltration, destruction of acini, irregularity of duct system similar to that seen in severe chronic inflammation (Stage II) of the lacrimal gland in Sjøgren's syndrome.

ASSOCIATION OF SJØGREN'S SYNDROME AND AUTO-IMMUNE THYROID DISEASE

Anderson, Gray, Beck and Kinnear (1961); Bunim (1961); Bloch and Bunim (1963); Anderson, Beck, Bloch, Buchanan and Bunim (1965), have shown that the prevalence of thyroglobulin autoantibodies is increased in patients with Sjøgren's syndrome, but the corresponding prevalence of Sjøgren's syndrome in patients with auto-immune thyroid disease has not been evaluated. The histological features of auto-immune thyroiditis (Hashimoto's thyroiditis, and spontaneous myxoedema) and those of the lacrimal and salivary glands in Sjøgren's syndrome are, however, very similar, as first noted by Hashimoto (1912) Fig.VII, 1 and 2. For these reasons it appeared to be important to determine whether the two conditions occur together with significant frequency. Since thyroid autoantibodies are also found in patients with thyrotoxicosis (Roitt and Doniach, 1958; Buchanan, Alexander, Crooks, Koutras, Wayne, Anderson and Goudie, 1961; Buchanan, Koutras, Crooks, Alexander, Brass, Anderson, Goudie and Gray, 1962; Anderson, Gray, Middleton and Young, 1964), the incidence of Sjøgren's syndrome in this disorder was also determined. In each group of patients the incidence of Sjøgren's syndrome was compared with that in a group with simple goitre and that in another control group of hospital patients.

Material and Methods

Patients

339 female patients comprising five groups were examined for keratoconjunctivitis sicca; mean age and age range are shown in Table VII,1.

Group 1 Hashimoto's Thyroiditis (83)

In five patients the diagnosis was based on histological examination of the gland using the criteria of Joll (1939) and in the remaining 78 on the presence of a positive precipitin test for antithyroglobulin autoantibodies in patients with a euthyroid or hypothyroid state (Buchanan and others, 1961). Three of these patients were hypothyroid, but the remaining eighty were receiving 0.2 mg. sodium thyroxine and were euthyroid when studied. Four had "definite" rheumatoid arthritis by the American Rheumatism Association criteria (Ropes, Bennett, Cobb, Jacox and Jessar, 1958).

Group 2 Spontaneous Primary Hypothyroidism (69) (hypothyroidism without a goitre)

The diagnosis was based on the clinical and laboratory criteria described by Wayne (1960) and Wayne, Koutras and Alexander (1964). Ten patients

were hypothyroid when examined and the remaining 59 were receiving 0.2-0.3 mg. sodium thyroxine and were euthyroid. Four had "definite" rheumatoid arthritis by the American Rheumatism Association criteria (Ropes and others, 1958).

Group 3 Thyrotoxicosis (68)

21 patients had become hypothyroid following radioactive iodine (^{131}I) therapy; twenty of them were euthyroid at the time of examination and were receiving 0.2-0.3 mg. sodium thyroxine per day and the other was hypothyroid. The remaining 47 were euthyroid following antithyroid drug therapy, subtotal thyroidectomy, or ^{131}I therapy.

Group 4 Simple Goitre (46)

All these were euthyroid, and none had systemic disease.

Group 5 Hospital Controls (72)

These were all women attending as outpatients at the clinics associated with the Western and Royal Infirmaries, Glasgow. They had a variety of general medical conditions, none of which, however, had any known association with thyroid disease or auto-immune disorders.

Methods

Autoantibodies to thyroglobulin were tested by a precipitin test using the Ouchterlony-Elekplate technique (Anderson, Buchanan, Goudie and Gray, 1962) and by the tanned red cell haemagglutination test using thyroglobulin-coated formalized tanned sheep red cells (Burroughs Wellcome), (Fulthorpe, Roitt, Doniach and Couchman, 1961). The lowest serum dilution used in the tanned red cell haemagglutination test was 1 in 16. Autoantibody to thyroid "microsomes" was measured by an immunofluorescence technique on unfixed frozen sections of thyroid slices (Holborow, Brown, Roitt and Doniach, 1959), using test serum diluted one in four in the first layer.

Sialography

This was performed in 109 patients, 41 with Hashimoto's thyroiditis, 32 with primary hypothyroidism, and 36 control subjects. A hydrostatic technique was employed, using Triosil "45" (sodium metrizoate) as a contrast medium. Apparatus consisting of a 20 ml. glass syringe, polythene tubing, and a tapered catheter was used to convey the contrast medium to the duct and gland. The glass syringe was set at a fixed height above the patient's head (70-90 cm.). The contrast medium flowed into the gland using only the force of gravity and therefore a relatively constant pressure

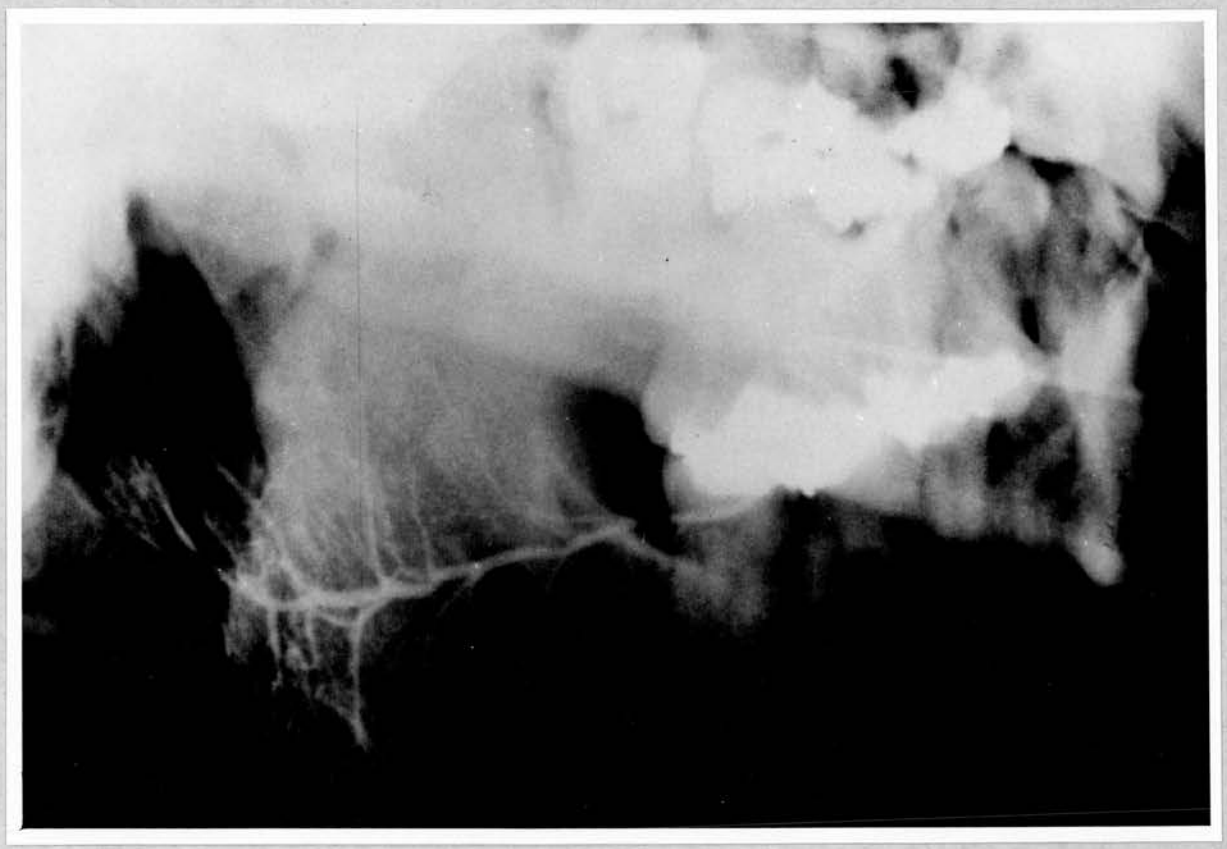

Fig. VII,3(a) Sialogram showing normal pattern during injection phase

Fig. VII,3(b) Punctate sialectasis

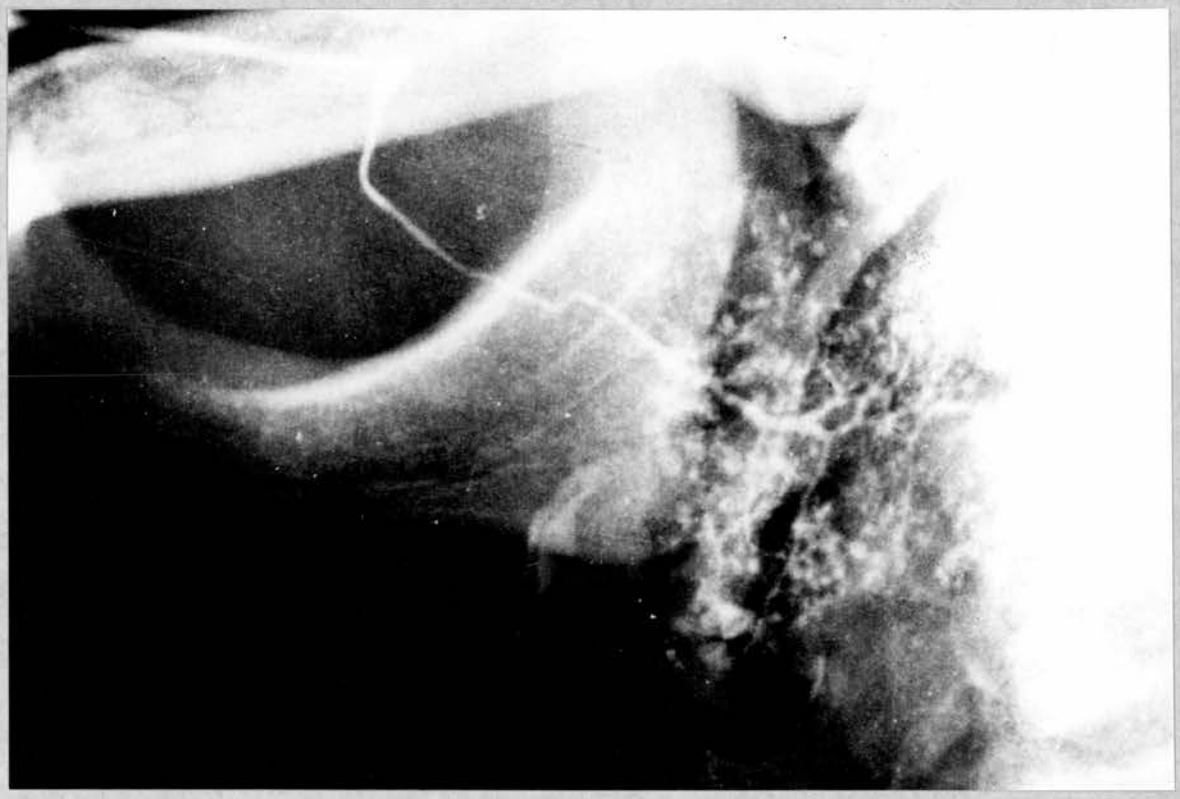

Fig. VII,3(c) Globular pattern injection phase

Fig. VII,3(d) Cavitary and destructive sialectasis

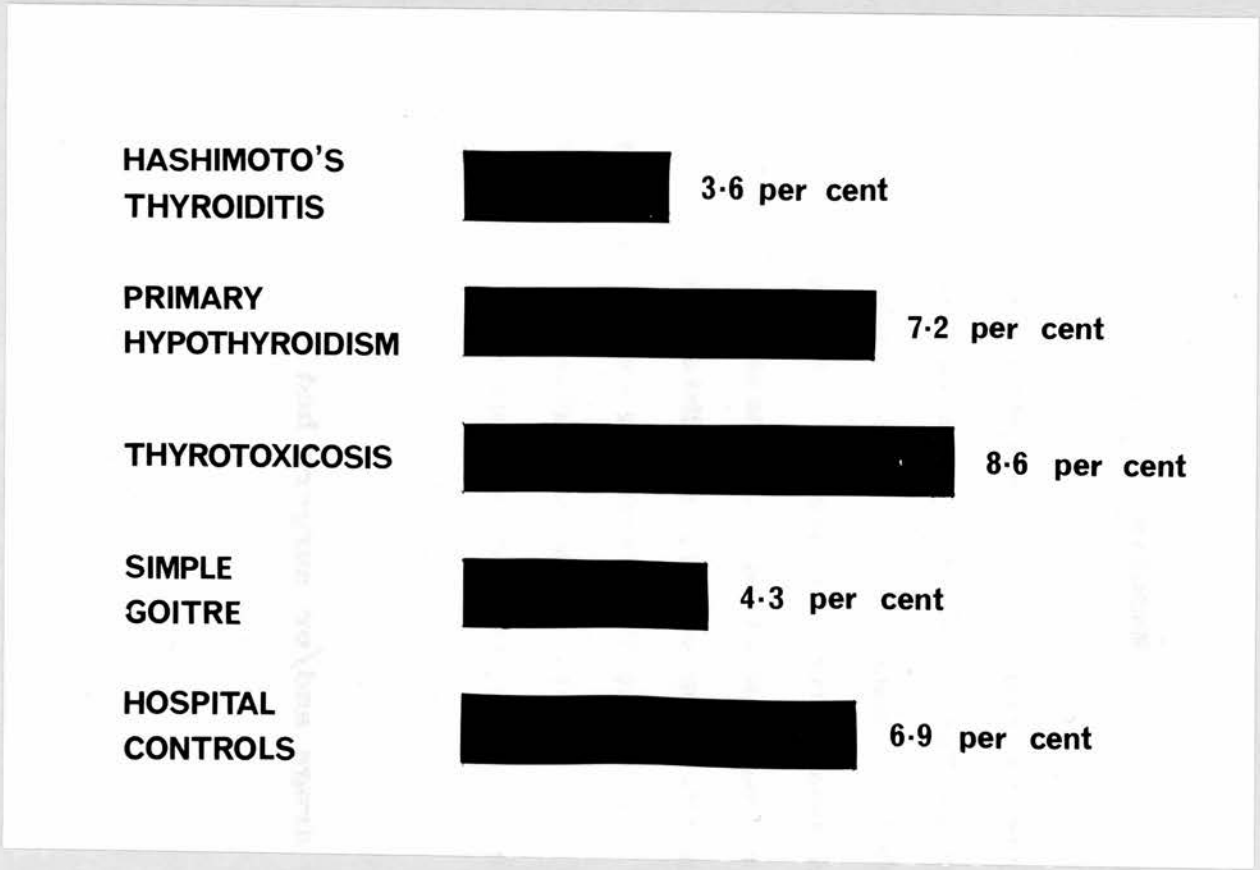

Fig. VII,4 Keratoconjunctivitis sicca in thyroid disease

was obtained (Park and Mason, 1966). This method rarely led to over-filling and was less painful for the patient. The water-soluble contrast medium is rapidly expelled from the gland and therefore a secretory phase film was exposed 5 minutes after the filling phase was complete. Between the two phases, the patient was asked to suck a slice of lemon to stimulate salivary flow (Fig.VII,3).

Results

The results of the ophthalmological examination are summarized in Table VII,1 and in histogram form in Fig.VII,4. Only a small minority in each group had definite keratoconjunctivitis sicca. The highest prevalence was found in Group 3, treated thyrotoxicosis (8.6 per cent), but this was not significantly different from that in Group 5, hospital controls (6.9 per cent). The 43 patients treated with ^{131}I therapy were reassessed separately (Table VII,2). Six of those with keratoconjunctivitis sicca came from this group and none of the remaining 25 treated by drugs and/or surgery had keratoconjunctivitis sicca. No correlation was, however, found between the presence of keratoconjunctivitis sicca and the number of doses, total dosage of ^{131}I, or the interval since the last dose of ^{131}I.

Clinical Groups	Number of Patients	Age (yrs)		Schirmer's Test (mm. at 5 minutes)						Kerato-conjunctivitis Sicca	
		Mean	Range	5		5–9		10–14		No.	Per cent.
				No.	Per cent.	No.	Per cent.	No.	Per cent.		
Hashimoto's Thyroiditis	83	55·0	37–75	5	6·0	11	13·3	7	8·4	3	3·6
Primary Hypothyroidism	69	67·0	33–74	6	8·7	12	17·3	5	7·2	5	7·2
Thyrotoxicosis	68	43·2	16–66	7	10·3	1	1·4	—	—	6	8·6
Simple Goitre	46	39·5	15–67	2	4·3	—	—	5	11·0	2	4·3
Hospital Controls	72	51·9	31–74	5	6·9	10	13·9	6	8·3	5	6·9

Table VII,1 Keratoconjunctivitis sicca in thyroid diseases

No. of Patients	No. of Doses of ^{131}I	Mean and Range of ^{131}I Doses (mc.)		No. with Kerato-conjunctivitis Sicca
		Mean	Range	
15	1	9·1	8–10	2
18	2	18·7	16–20	3
10	3	29·1	24–30	1

Table VII,2 Relationship between ^{131}I therapy in thyroid disease and keratoconjunctivitis sicca

Clinical Group	No. of Patients	Sialographic Abnormalities					No. with Abnormal Sialograms and Xerostomia	No. with Abnormal Sialograms and Salivary Gland Enlargement
		Total		Punctate	Punctate with Intermediate Duct Changes	Globular		
		No.	Per cent.					
Hashimoto's Thyroiditis	41	7	16	3	2	2	6	0
Primary Hypothyroidism	32	5	16	3	2		4	0
Hospital Controls	36	6	16	4	2		6	0

Table VII,3 Sialographic abnormalities in thyroid disease

None of the 83 patients with Hashimoto's thyroiditis and none of the four with primary hypothyroidism plus rheumatoid arthritis had keratoconjunctivitis sicca. None of the five patients with Hashimoto's thyroiditis in whom the diagnosis was confirmed by biopsy had keratoconjunctivitis sicca.

Abnormal sialograms were found in eighteen cases: seven (16 per cent) of the 41 patients with Hashimoto's thyroiditis, five (16 per cent) of the 32 with primary hypothyroidism, and in six (16 per cent) of the 36 in the hospital control group (Table VII,3). Only two patients with Hashimoto's thyroiditis showed globular sialectasis, the remaining patients having only minor abnormalities consisting of punctate sialectasis with or without intermediate duct changes. Mild xerostomia was found in sixteen of these eighteen patients. None had a history or clinical evidence of salivary gland enlargement. Two of the hospital control subjects had unexplained xerostomia but normal sialograms. None of the patients with Hashimoto's thyroiditis or with primary myxoedema who had rheumatoid arthritis had xerostomia or abnormal sialograms.

Discussion

This study shows no increased prevalence of keratoconjunctivitis sicca in patients with proven auto-immune thyroid disease (Table VII,1). The

prevalence is higher than in Sjøgren's original series of 19 cases which were distributed among 36,000 hospital patients (Sjøgren, 1933) and de Roetth's group of ophthalmic control patients where there was 0.2 per cent of cases in 6,200 patients (de Roetth, 1945). However, in neither series was the age and sex distribution of the patients recorded.

The number of patients with xerostomia and sialographic abnormalities consistent with Sjøgren's disease affecting the parotid glands was also no higher in the groups with Hashimoto's thyroiditis and primary hypothyroidism respectively than in the hospital control group (Table VII,3).

The prevalence of keratoconjunctivitis sicca in patients with thyrotoxicosis (8.6 per cent) was higher, but not significantly higher, than in the patients with simple goitre (4.3 per cent) and the hospital controls (6.9 per cent). 64 per cent of the patients with thyrotoxicosis received treatment with ^{131}I therapy, but no correlation was found between the number of doses or the total amount given. It does not appear, therefore, that radioactive iodine results in subsequent irradiation damage to the lacrimal and accessory lacrimal glands of the eye. It is of interest to note, however, that irradiation parotitis and xerostomia have been noted in patients receiving similar doses of ^{131}I therapy for thyrotoxicosis (Chapman and Maloof, 1955).

The prevalence of thyroglobulin autoantibodies in

patients with Sjøgren's syndrome is, however, increased (Anderson and others, 1961; Bunim, 1961; Bloch and Bunim, 1963; Anderson and others, 1965), and thyroglobulin autoantibodies have also been reported to occur with increased frequency in the connective tissue diseases, rheumatoid arthritis (Anderson and others, 1961; Bloch and others, 1965), and systemic lupus erythematosus (Anderson and others, 1961; Hijmans, Doniach, Roitt and Holborow, 1961), all of which may be associated with established keratoconjunctivitis sicca. The absence of an increased prevalence of keratoconjunctivitis sicca in auto-immune thyroid disorders in contrast to that in auto-immune systemic disorders is consistent with the concept that auto-immune thyroiditis is an organ specific disorder.

Summary

83 patients with Hashimoto's thyroiditis, 69 with primary hypothyroidism, and 68 with thyrotoxicosis were examined for keratoconjunctivitis sicca. The prevalence of keratoconjunctivitis sicca in these patients was no higher than in 46 patients with simple goitre and in 72 hospital controls matched for age and sex. Sialography was performed in 41 patients with Hashimoto's thyroiditis, 23 with primary hypothyroidism, and 36 of the hospital controls. Sialographic abnormalities suggestive of Sjogren's syndrome were

found as frequently in the hospital controls as in the patients with Hashimoto's thyroiditis and primary hypothyroidism.

Association of Sjøgren's Syndrome with Pernicious Anaemia and Idiopathic Addison's Disease

Lymphocytic infiltration of the gastric mucosa in chronic gastritis is associated with the development of pernicious anaemia in a proportion of cases (Anderson, Buchanan and Goudie, 1967). More than 40 per cent of patients suffering from pernicious anaemia can be shown to have antibodies to gastric parietal cells (Irvine, Davies, Delamore and Williams, 1962; Markson and Moore, 1962; Taylor, Roitt, Doniach, Couchman and Shapland, 1962; Irvine, 1963). It occurred to the writer that the lymphocytic infiltration in chronic gastritis might be similar to that found in the lacrimal and salivary glands in Sjøgren's syndrome and it is noted that a series of patients suffering from this disease was found to have a high incidence of autoantibodies to gastric parietal cells (Buchanan, Cox, Harden, Glen, Anderson and Gray, 1966). Further, patients suffering from pernicious anaemia have a high incidence of thyroglobulin antibodies (Irvine and others, 1962; Markson and Moore, 1962; Taylor and others, 1962; Doniach, Roitt and Taylor, 1963) as do patients with Sjøgren's syndrome (Anderson, Goudie, Gray and Buchanan, 1961; Bloch, Buchanan, Wohl and Bunim, 1965).

It was, therefore, important to determine the incidence of Sjøgren's syndrome in patients suffering from proven pernicious anaemia.

In idiopathic Addison's disease there is atrophy of both adrenal cortices with loss of most of the cortical cells, lymphocytic and plasma cell infiltration and minimal fibrosis. Chronic thyroiditis, which demonstrates similar histological changes, is present in approximately 50 per cent of patients with idiopathic Addison's disease examined at post mortem (Wells, 1930; Sloper, 1953; Bloodworth, Kirkendall and Carr, 1954). Primary myxoedema has occurred with idiopathic Addison's disease with sufficient frequency to warrant the term Schmidt's syndrome (Schmidt, 1926). In addition, the chronic thyroiditis found in idiopathic Addison's disease is associated with thyroid microsomal antibodies in about 30 per cent of the patients studied (Blizzard and Kyle, 1963; Irvine, 1963) and thus is probably of the auto-immune type. Pernicious anaemia has been reported in idiopathic Addison's disease (Blizzard and Kyle, 1963; Irvine, 1963; Kra and Barile, 1964). Gastric mucosa biopsies reveal a high incidence of chronic gastritis in patients with idiopathic Addison's disease (Feyrter and Klima, 1952; Smith, Delamore, Wynn and Williams, 1961). Furthermore, gastric parietal cell antibodies are more prevalent in idiopathic Addison's disease than in tuberculous cases (Irvine, 1963). There is, therefore, strong evidence, clinical, histological and immunological, pointing to an association between idiopathic Addison's disease, chronic thyroiditis and chronic gastritis (which of course predisposes to pernicious anaemia).

Antibodies to salivary duct epithelium have been reported in patients with idiopathic Addison's disease (Blizzard and Kyle, 1963) and similar antibodies have been detected in Sjøgren patients (Bertram and Halberg, 1964; Halberg, Bertram, Søborg and Nerup, 1965; MacSween, Goudie, Anderson, Armstrong, Murray, Mason, Jasani, Boyle, Buchanan and Williamson, 1967).

The second purpose of this investigation, therefore, was to determine the prevalence of Sjøgren's syndrome in patients suffering from idiopathic Addison's disease.

Materials and Methods

Patients

169 patients (120 female, 49 male) comprising three groups, pernicious anaemia, idiopathic Addison's disease and hospital controls, were included in the survey (the mean age and age range are shown in Table VII,4). All of the patients were examined for evidence of keratoconjunctivitis sicca. The patients in the first two groups had been investigated as inpatients. Together with the help of the original case records and further specific examinations, evidence of rheumatoid arthritis, thyroid disease and salivary duct atrophy was collected. Most of the hospital control patients were attending as outpatients and were not suffering from any disease known to have an auto-immune basis.

Group 1

77 patients (40 female, 37 male) had pernicious anaemia. Four of these had rheumatoid arthritis by the American Rheumatism Association criteria (Ropes, Bennett, Cobb, Jacox and Jessar, 1958). Two patients had Hashimoto's thyroiditis (Buchanan, Alexander, Crooks, Koutras, Wayne, Anderson and Goudie, 1961) and one idiopathic Addison's disease (Anderson, Buchanan and Goudie, 1967). One patient had thyrotoxicosis as well as rheumatoid arthritis and another had thyrotoxicosis.

Group 2

Idiopathic Addison's Disease, (primary adrenal atrophy) (20 patients, 15 male, 5 female). The diagnosis was based on the exclusion of obvious causes, e.g. tuberculosis for extensive and irreversible destruction of the cortex of the adrenal glands (Anderson, and others, 1967) and on the detection of antibodies to adrenal cortical cells (Anderson, Goudie, Gray and Timbury, 1957; Blizzard and Kyle, 1963; Goudie, Anderson, Gray and Whyte, 1966).

Group 3

Hospital Controls (72). These were female patients attending clinics associated with the Western and Royal Infirmaries, Glasgow. None of the variety of general

medical conditions from which they suffered had any known association with pernicious anaemia, thyroid disease or auto-immune disorders.

Methods

Antibodies to Parietal Cells were tested using the indirect immunofluorescence technique for the detection of gastric autoantibodies. Antigen was prepared from frozen unfixed sections of normal gastric fundal mucosa (Taylor and others, 1962).

Autoantibodies to Thyroglobulin were tested by a precipitin test using the Ouchterlony-Elekplate technique (Anderson, Buchanan, Goudie and Gray, 1962) and by tanned red cell haemagglutination test using thyroglobulin-coated formalized tanned sheep red cells (Burroughs Wellcome), (Fulthorpe, Roitt, Doniach and Couchman, 1961). Autoantibody to thyroid microsomes was measured by an immunofluorescence technique on unfixed frozen sections of thyroid slices (Holborow, Brown, Roitt and Doniach, 1959).

Other Laboratory Data

Rose Waaler, Latex particle and haemoglobin tests were recorded in the patients suffering from pernicious anaemia.

Sialography was performed on four patients who gave a history of dryness of the mouth and throat and who had clinical xerostomia (Park and Mason, 1966).

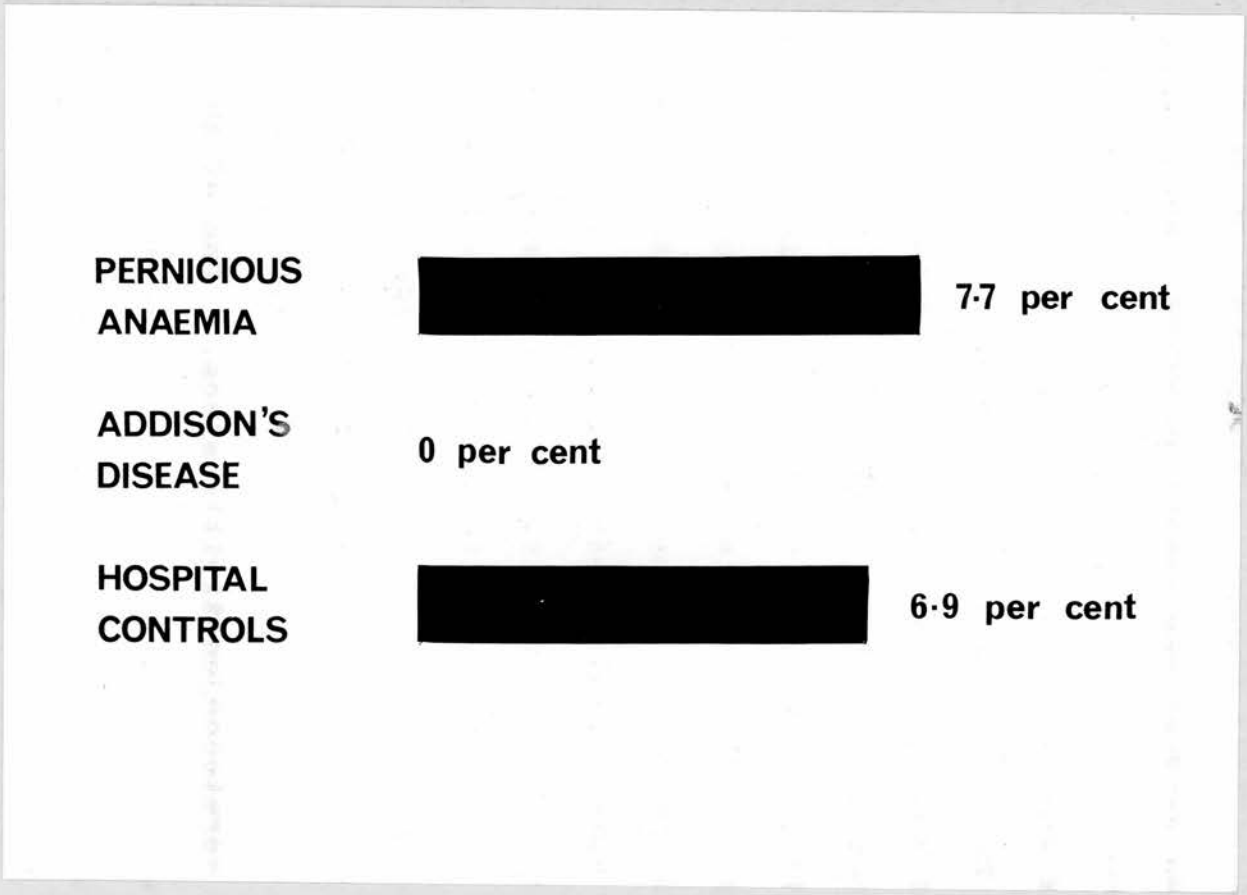

Fig. VII,5 Keratoconjunctivitis sicca in pernicious anaemia and Addison's disease

Results

The results of the examination for keratoconjunctivitis sicca are summarised in Table VII,4 and in histogram form, Fig.VII,5. A small number of patients in Group 1, pernicious anaemia, and Group 3, hospital controls, were suffering from keratoconjunctivitis sicca. Although the incidence of the ocular disease was higher in Group 1 than Group 3 the difference was not significant.

None of the patients with idiopathic Addison's disease had keratoconjunctivitis sicca.

Four of the patients with pernicious anaemia had xerostomia but normal sialograms.

Table VII,5 summarises the principal findings in those patients who had pernicious anaemia and evidence of Sjøgren's syndrome. Four of the patients had evidence of involvement in other diseases. Patient one had rheumatoid arthritis, patient two xerostomia and Hashimoto's thyroiditis, patient three Hashimoto's thyroiditis and idiopathic Addison's disease and patient four xerostomia. Three other patients had pernicious anaemia and rheumatoid arthritis but no evidence of keratoconjunctivitis sicca. One of the patients with pernicious anaemia and rheumatoid arthritis also suffered from thyrotoxicosis.

Clinical groups	*No. of patients*	*Age (yrs)*		*Schirmer's test (mm. at 5 minutes)*						*Kerato-conjunc-tivitis sicca*	
		Mean	*Range*	5		5–9		10–14			
				No.	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*
Pernicious anaemia	77	62·5 ± 11·1	20–82	11	14·1	14	18·0	12	14·2	6	7·7
Addison's disease	20	36·6 ± 6	20–45	—	—	—	—	1	5·0	—	—
Hospital controls	72	51·9	31–74	5	6·9	10	13·9	6	8·3	5	6·9

Table VII,4 Keratoconjunctivitis sicca in pernicious anaemia and Addison's disease

Patient no.	*Pernicious anaemia*	*Kerato-conjunctivitis sicca*	*Xerostomia*	*Rheumatoid arthritis*	*Hashimoto's thyroiditis*	*Idiopathic Addison's disease*
1	+	+	o	+	o	o
2	+	+	+	o	+	o
3	+	+	o	o	+	+
4	+	+	+	o	o	o
5	+	+	o	o	o	o
6	+	+	o	o	o	o
7	+	o	+	o	o	o
8	+	o	+	o	o	o

Table VII,5 Summary of coexisting disease in the 8 patients suffering from pernicious anaemia and keratoconjunctivitis sicca

Other Investigations

A family history of pernicious anaemia was obtained from 14 of the 77 patients (18.2 per cent) suffering from this disease and a history of thyroid disorders in 8 (10.4 per cent). There was no history of pernicious anaemia or thyroid disease in the families of those suffering from idiopathic Addison's disease.

25 of the 77 pernicious anaemia patients (32.4 per cent) had palpable thyroid glands. In 20 patients the glands were soft, in 5 firm. Five of the patients with pernicious anaemia had proven thyroid disease; two had Hashimoto's thyroiditis, two thyrotoxicosis and one primary myxoedema.

Laboratory Data

All of the patients with pernicious anaemia were receiving Cytamen injections, the current mean haemoglobin being 11.5 $\pm$ 2.1 gm./100 ml. Antibodies to gastric parietal cells were detected in the sera of 35 of 46 patients (77 per cent), antibodies to thyroid microsomes in 18 of 46 patients (39 per cent) and anti-thyroglobulin antibodies were observed in 13 of 46 patients (28 per cent) suffering from pernicious anaemia.

Discussion

This study, like the previous one concerning Sjøgren's syndrome and thyroid disease, shows no increased prevalence of keratoconjunctivitis sicca in patients suffering from pernicious anaemia (Table VII,4). Once again the incidence is higher than in Sjøgren's (1933) and de Roetth's (1945) series but in neither of these was the age and sex distribution recorded. Some authorities believe that a patient with any one organ specific disease has a higher than normal tendency to develop another organ specific disorder (Anderson, and others, 1967). In this series it is interesting to observe that two of the six patients with pernicious anaemia and keratoconjunctivitis sicca also had auto-immune thyroiditis - Hashimoto's disease (Table VII,5) and one of them in addition had idiopathic Addison's disease.

However, the inability of the present investigation to show an increased prevalence of keratoconjunctivitis sicca in pernicious anaemia or thyroid disease (v.s.) would tend to contradict this concept. Furthermore, the number of patients with xerostomia was no higher than in the hospital control group. One of the pernicious anaemia patients who had keratoconjunctivitis sicca was also suffering from rheumatoid arthritis. The remaining three patients with rheumatoid arthritis and pernicious anaemia did not have Sjøgren's syndrome.

No instances of Sjøgren's syndrome were detected

in the 20 patients who had a primary diagnosis of idiopathic Addison's disease. Their mean age, 36.6 years, is much younger than either the pernicious anaemia group or the hospital control patients. However, as described above, one patient in the pernicious anaemia series had idiopathic Addison's disease and keratoconjunctivitis sicca.

Nevertheless, the prevalence of gastric parietal cell antibodies and thyroglobulin antibodies is increased in Sjogren's syndrome (Buchanan, Cox and others, 1966; Anderson and others, 1961; Bloch and others, 1965) and both of these antibodies occur with increased frequency in pernicious anaemia (Irvine and others, 1962; Markson and Moore, 1962; Taylor and others, 1962; Irvine, 1963; Doniach and others, 1963) and in idiopathic Addison's disease (Blizzard and Kyle, 1963; Irvine, 1963). Thyroglobulin antibodies are more frequent in the connective tissue diseases, rheumatoid arthritis (Anderson and others, 1961; Bloch and others, 1965) and systemic lupus erythematosus (Anderson and others, 1961; Hijmans, Doniach, Roitt and Holborow, 1961) both of which may be associated with established keratoconjunctivitis sicca. The absence of an increased prevalence of keratoconjunctivitis sicca in pernicious anaemia or in idiopathic Addison's disease in contrast to that in auto-immune systemic disorders is consistent with the concept that pernicious anaemia and idiopathic Addison's disease are organ specific disorders.

Summary

77 patients suffering from pernicious anaemia and 20 patients with idiopathic Addison's disease were examined for keratoconjunctivitis sicca. The prevalence of keratoconjunctivitis sicca in these patients was no higher than in 72 hospital control patients. Sialography was performed on 4 patients who had xerostomia but no abnormalities were detected.

SUMMARY

The histological features of the lacrimal and salivary glands in Sjøgren's syndrome are reminiscent of those in the thyroid gland in Hashimoto's thyroiditis and primary myxoedema. Furthermore, the prevalence of thyroglobulin autoantibodies is high in patients suffering from Sjøgren's syndrome, Hashimoto's thyroiditis, primary myxoedema and thyrotoxicosis. However, this study has failed to demonstrate any increased prevalence of Sjøgren's syndrome in patients suffering from thyroid disease.

Similarly, it may be argued that the lymphocytic infiltration of the gastric mucosa in chronic gastritis is not unlike that in the lacrimal and salivary glands in Sjøgren's syndrome. Patients with chronic gastritis have a higher than normal tendency to develop pernicious anaemia and conversely patients with pernicious anaemia nearly always suffer from chronic gastritis. The prevalence of gastric parietal cell antibodies is high in pernicious anaemia and according to some authorities in Sjøgren's syndrome. In addition, patients suffering from pernicious anaemia have, like Sjøgren patients, a high incidence of thyroglobulin autoantibodies. However, when a series of patients suffering from pernicious anaemia was examined for Sjøgren's syndrome no increased prevalence was detected.

Patients suffering from idiopathic Addison's disease appear to have an increased susceptibility to

chronic thyroiditis associated with thyroid microsomal antibodies. Pernicious anaemia has also been reported in this disease and the prevalence of gastric parietal cell antibodies is higher than in tuberculous cases. Salivary duct antibodies have been reported in idiopathic Addison's disease and Sjøgren's syndrome. On the other hand, a series of patients suffering from idiopathic Addison's disease was examined for increased prevalence to Sjøgren's syndrome without success.

The results of these studies are consistent with the concept that auto-immune thyroiditis, pernicious anaemia and idiopathic Addison's disease are organ specific disorders. If keratoconjunctivitis sicca and/or xerostomia is diagnosed in the absence of a generalised connective tissue disease then it may be regarded as an organ specific auto-immune disorder and the belief exists that patients suffering from one organ specific disease are more susceptible to the development of another organ specific disease. The failure of these investigations to demonstrate any increased prevalence of Sjøgren's syndrome in auto-immune thyroid disease, pernicious anaemia or idiopathic Addison's disease tends to contradict this thesis.

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CHAPTER VIII

STUDIES OF THE BACTERIAL, FUNGAL AND VIRAL FLORA IN KERATOCONJUNCTIVITIS SICCA

Keratoconjunctivitis sicca is a chronic disorder, that behaves erratically, being non-progressive in some patients whilst proceeding in extreme cases, sometimes with alarming speed, to perforation of the cornea and blindness.

In this chapter the frequency and identity of bacteria, fungi and viruses in the dry eye are recorded and their possible significance in relation to the ocular complications of Sjøgren's syndrome is examined.

STUDIES OF THE BACTERIAL FLORA IN KERATOCONJUNCTIVITIS SICCA

A great deal of attention has been given to the bacterial flora of the conjunctival sac in health and disease and lengthy investigations have been carried out in many centres including the United Kingdom (Smith, 1954; Soudakoff, 1954; Orfila and Courden, 1961). Views vary as to how often the healthy sac is devoid of organisms and what effect climate, hygiene, coincidental disease and the use of topical antibiotics may have on the range of bacteria detected. Nevertheless, there is general agreement about the commonest bacterial organisms found in the healthy sac, the inhibitory effect of lysozyme and the mechanical effect of the lacrimal secretions (Duke Elder, 1965). Since both lysozyme content (Thygeson and Kimura, 1963) and tear secretion (Sjøgren, 1933) are reduced in keratoconjunctivitis sicca, it is important to investigate the nature of the bacterial flora in the conjunctival sac of the dry eye.

During the process of collection data for the diagnosis of keratoconjunctivitis sicca, I noticed that quite a number of the patients had evidence of blepharitis. For this reason the prevalence of bacteria on the lid margins was also examined. Nasal carriers of bacteria, particularly of staphylococcus coagulase positive organisms are regarded as potentially infectious and a great deal of energy has been expended in determining

the incidence of carriers in hospital and non-hospital patients (Rogers, Duffy and Mou, 1965; Polakoff, Richards, Parker and Lidwell, 1967; Noble, Valkenburg and Wolters, 1967). It is generally accepted that the nose if the most frequent site of carriage for staphylococcus coagulase positive bacteria in man (Polakoff, Richards, Parker and Lidwell, 1967) and nasal carriers of pathogenic staphylococci have a higher isolation rate for the organism on their hands than do non-carriers (Noble, Valkenburg and Wolters, 1967). Consequently, the bacterial flora in the anterior nares of the patients suffering from keratoconjunctivitis sicca were also examined.

Material and Methods

Patients

1. Clinically healthy conjunctival sacs, lid margins and anterior nares. Sixty (60) arthritic patients comprising 5 males and 55 females, without clinical evidence of external ocular inflammation, were selected for the investigation of bacterial flora in the healthy conjunctival sac, lid margins and anterior nares. All of them were suffering from rheumatoid arthritis and had been referred over a period of 2 years for routine ophthalmological examination. Their mean age was 55.2 years ($\pm$ 11.1 standard deviation).

2 Untreated Keratoconjunctivitis Sicca

(a) The association of bacterial colonisation and the dry eye was studied in 65 patients diagnosed over a period of three years. The group consisted of 8 males and 57 females whose mean age was 57.7 years (standard deviation ± 9.8). 8 patients had been treated by their general practitioners for chronic conjunctivitis and 10 patients had been in the habit of bathing their eyes with a variety of commercially obtainable solutions, such as Optrex and Ocusol. However, none of the patients had received any topical therapy for at least 8 weeks before they were included in the study.

All of the bacterial investigations were repeated after one month of tear substitute therapy.

(b) When the diagnosis of keratoconjunctivitis sicca was made, a record of objective evidence suggesting bacterial infection of the external eye was compiled with a view to relating bacterial isolates to the clinical state of the patients' eyes.

Collection of Specimens

In all cases, specimens for culture were taken from both conjunctival sacs using sterilised cotton tipped sticks with which the lower fornices were scraped. The same technique was applied to the lower

Bacterial flora from the conjunctival sacs of 60 patients with clinically healthy eyes.

Bacteria isolated	PATIENTS		Percentage of total isolates
	Number	Per cent	
Staphylococcus (all types)	16	26.6	36.3
Staphylococcus coagulase positive	6	10	13.6
Diphtheroids	14	22.3	31.8
Neisseria Catarrhalis	5	8.3	11.3
Pneumococcus	2	3.2	4.5
Haemolytic Streptococcus	1	1.6	2.2
Streptococcus viridans	4	6.6	9.1
Escherichia Coli	2	3.2	4.5
Sterile sacs	24	40	-

Table VIII,1

Bacterial flora from the lid margins of 60 patients with clinically healthy eyes.

Bacteria isolated	Patients		Percentage of total isolates
	Number	per cent	
Staphylococcus (all types	25	41.6	41.5
Staphylococcus coagulase positive	9	15	14.7
Diphtheroids	10	16.6	16.4
Neisseria Catarrhalis	2	3.3	3.3
Pneumococcus	3	5	5
Haemolytic Streptococcus	3	5	5
Streptococcus Viridans	4	6.7	6.6
Escherichia Coli	2	3.3	3.3
Proteus	1	1.6	1.6
Haemophilus Influenza	2	3.3	3.3
Sterile	18	30	-

Table VIII,2

lid margins and to one anterial nasal vestibule. Cultures were also taken at irregular intervals from the mydriatics, miotics, vital dyes and tear substitutes in current use at the Outpatient Departments.

Cultivation of Bacteria

All specimens were inoculated within two hours on to blood agar plates and incubated at 37°C. The filter paper disc technique was used for testing sensitivity employing dishes saturated in the variety of antibiotics listed below.

Penicillin, ampicillin, cloxacillin, tetracycline, chloromycetin, erythromycin and latterly septrin, neomycin.

Results

1 Clinically healthy conjunctivae, lid margins and anterior nares. Tables VIII,1,2 and 3 show the frequency and identity of bacterial isolates from the conjunctival sacs, lid margins and anterior nares of 60 arthritic patients, none of whom had any evidence of external ocular disease. Bacteria were isolated from the conjunctival sacs of 36 patients (60 per cent) and from the lid margins of 42 patients (70%), a difference which is not statistically significant. Staphylococci

Bacterial flora from the anterior nares of 60 patients with clinically healthy eyes.

Bacteria isolated	Patients		Percentage of total isolates
	Number	Per cent	
Staphylococcus (all types)	31	51.6	64.5
Staphylococcus coagulase positive	14	23.3.	29.1
Diphtheroids	9	15.0	18.7
Pneumococcus	2	3.2	4.1
Haemolytic Streptococcus	3	5.0	4.1
Escherichia Coli	3	5.0	6.2
Sterile nares	20	33.3	-

Table VIII,3

and diphtheroids were the commonest organisms detected in both sites and staphylococcus coagulase positive (staphylococcus aureus) was the commonest pathogen. Thus, staphylococcus aureus was isolated from conjunctival sac of 6 patients (10 per cent) and the lid margins of 9 patients (15 per cent), isolation rates which were not significantly different from one another. However, if all of the bacteria, normally regarded as pathogens are summated then their prevalence on lid margins is higher than in conjunctival sacs, although once again the probability of this being significant is doubtful (9 of 60 patients with sac isolated, 17 of 60 patients with lid isolates, $X^2 = 2.691$, $P < 0.1$). Bacterial isolates were obtained from the anterior nares of 40 patients (66.6 per cent) only two less than the number of patients with lid isolates. Staphylococcus aureus represented a larger percentage of the total isolates from the nose than from the lid margins or the conjunctival sacs but only in the case of the latter may the difference have some significance ($X^2 = 2.7518$, $P < 0.1$).

Coagulase positive staphylococci were isolated from the conjunctival sacs of 6 patients (Table VIII,1) and, in addition, from the lid margins and anterior nares of one patients and the lid margins of two patients. A fourth patient had the organisms on her lid margins and anterior nares but not the conjunctival sac. The only other pathogen found from more than one site in the same patient was the pneumococcus which appeared in the sacs

Bacterial flora in the conjunctival sacs of 65 patients suffering from untreated keratoconjunctivitis sicca.

Bacteria isolated	Patients		Per centage of total isolates
	Number	Per cent	
Staphylococcus (all types)	35	53.9	50.0
Staphylococcus coagulase positive	29	44.6	41.3
Diphtheroids	18	27.9	25.7
Neisseria Catarrhalis	3	4.6	4.2
Pneumococcus	4	6.1	5.7
Haemolytic streptococcus	2	3.2	2.8
Streptococcus viridans	3	4.6	4.2
Escherichia coli	2	3.2	2.8
Haemophilus aegyptius (Koch (Week's Bacillus)	1	1.6	1.4
Proteus	2	3.2	2.8
Sterile sacs	6	9.2	-

Table VIII,4

Bacterial flora on the lid margins of 65 patients suffering from untreated keratoconjunctivitis sicca.

Bacteria Isolated	Patients		Percentage of total isolates
	Number	Per cent	
Staphylococcus (all types)	45	66.6	41.2
Staphylococcus coagulase-positive	38	59.0	38.4
Diphtheroids	11	16.9	10.1
Pneumococcus	4	6.1	3.6
Haemolytic streptococcus	3	4.6	2.7
Streptococcus viridans	3	4.6	2.7
Escherichia coli	3	4.6	2.7
Proteus	2	3.0	1.8
Sterile	3	6.1	-

Table VIII,5

Bacterial flora from the anterior nares of 65 patients with untreated keratoconjunctivitis sicca.

Bacteria isolated	Patients		Percentage of total isolates
	Number	Per cent	
Staphylococcus (all types)	32	49.7	72.7
Staphylococcus coagulase-positive	16	24.6	36.4
Diphtheroids	5	7.6	11.3
Pneumococcus	2	3.0	4.5
Haemolytic streptococcus	1	1.5	2.2
Streptococcus viridans	3	4.6	6.8
Escherichia coli	1	1.5	2.2
Sterile nares	22	33.8	-

Table VIII,6

and lid margins of one patient.

2 Untreated Keratoconjunctivitis Sicca

(a) The bacterial flora in the conjunctival sacs, lid margins and anterior nares of 65 patients suffering from untreated keratoconjunctivitis sicca are shown in Tables VIII,4,5 and 6. Although the prevalence of bacteria in the conjunctival sacs and lid margins of these patients appears to be considerably higher than in patients with healthy eyes (Figure VIII,1) the differences are not in fact significant (X^2 = 2.2410 and 1.3327). However, staphylococcus aureus was detected in the conjunctival sacs of 29 patients (44.6 per cent) with untreated keratoconjunctivitis sicca, compared with only 6 (10 per cent) with clinically healthy eyes (Figure VIII,2) a difference which is highly significant (X^2 = 10.7432, $P < 0.01$). Similarly, coagulase positive organisms were significantly more prevalent on the lids of 38 patients (59 per cent) with keratoconjunctivitis sicca than in 9 rheumatoid patients (15 per cent) with healthy eyes (X^2 = 11.8351, $P < 0.001$).

The identity and frequency of bacterial isolates from the anterior nares of the two groups of patients are not significantly different (Tables VIII,3 and 6, Figures VIII,1 and 2). Coagulase positive staphylococci were isolated more frequently from the nose than from the conjunctival sacs or lid margins of patients with healthy eyes. The reverse applies to the

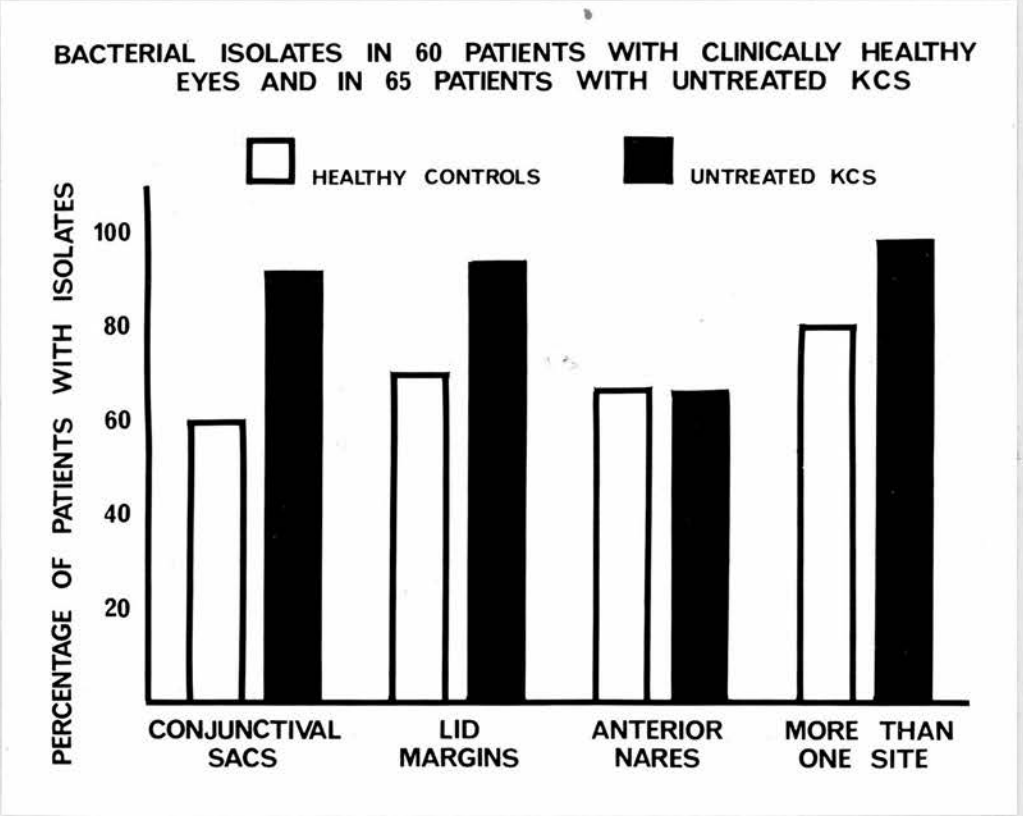
BACTERIAL ISOLATES IN 60 PATIENTS WITH CLINICALLY HEALTHY EYES AND IN 65 PATIENTS WITH UNTREATED KCS
HEALTHY CONTROLS
UNTREATED KCS
PERCENTAGE OF PATIENTS WITH ISOLATES
100
80
60
40
20
CONJUNCTIVAL SACS
LID MARGINS
ANTERIOR NARES
MORE THAN ONE SITE

Fig.VIII,1

COAGULASE POSITIVE STAPHYLOCOCCI IN 60 PATIENTS WITH CLINICALLY HEALTHY EYES AND IN 65 PATIENTS WITH UNTREATED KERATOCONJUNCTIVITIS SICCA

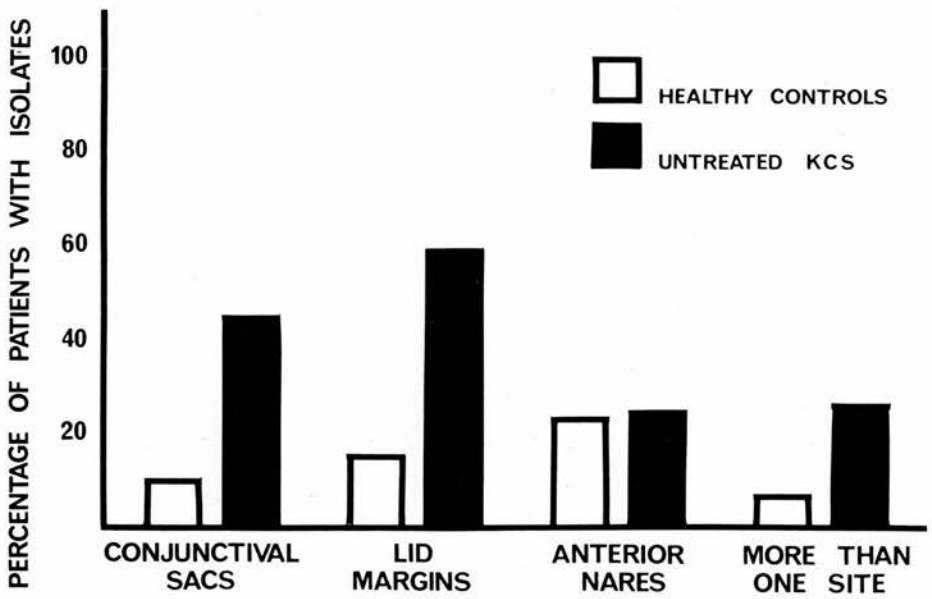

Fig.VIII,2

Bacterial flora in conjunctival sacs of 65 patients with keratoconjunctivitis sicca after one month of tear substitute therapy.

Bacteria isolated	Patients		Percentage of total isolates
	Number	Per cent	
Staphylococcus (all types)	25	39.0	62.5
Staphylococcus coagulase-positive	15	23.0	37.5
Diphtheroids	10	15.3	25
Neisseria Catarrhalis	2	3.1	5
Streptococcus viridans	3	4.6	7.5
Sterile sacs	30	46.1	-

Table VIII,7

Bacterial flora on lid margins of 65 patients with keratoconjunctivitis sicca after one month of tear substitute therapy.

Bacteria isolated	Patients		Percentage of total isolates
	Number	Per cent	
Staphylococcus (all types)	30	46.1	69.8
Staphylococcus coagulase-positive	23	35.3	53.5
Diphtheroids	9	13.8	20.9
Haemolytic streptococcus	2	3.1	4.6
Streptococcus viridans	2	3.1	4.6
Sterile	26	40	-

Table VIII,8

Bacterial flora in the anterior nares of 65 patients with keratoconjunctivitis sicca after one month of tear substitute therapy.

Bacteria isolated	Patients		Percentage of total isolates
	Number	Per cent	
Staphylococcus (all types)	34	52.3	77.1
Streptococcus coagulase-positive	15	23.1	34.1
Diphtheroids	6	9.2	13.6
Pneumococcus	1	1.5	2.2
Streptococcus viridans	3	4.6	6.8
Sterile nares	24	36.9	-

Table VIII,9

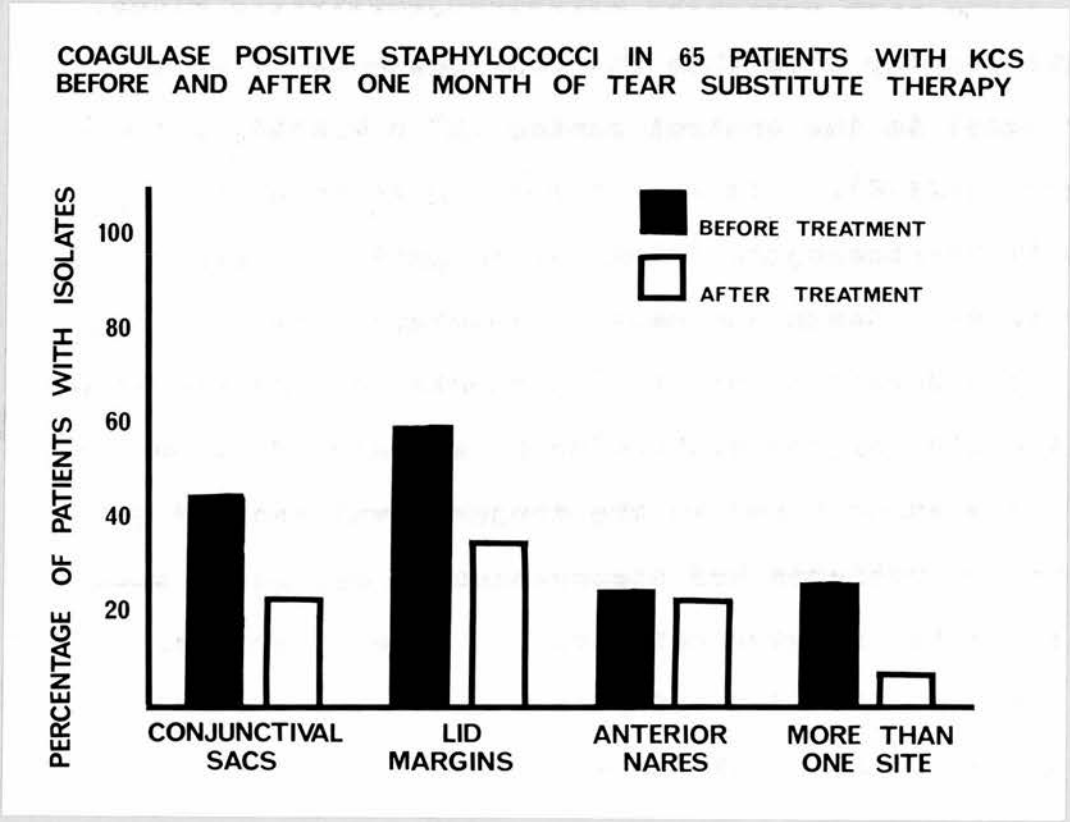
COAGULASE POSITIVE STAPHYLOCOCCI IN 65 PATIENTS WITH KCS
BEFORE AND AFTER ONE MONTH OF TEAR SUBSTITUTE THERAPY
PERCENTAGE OF PATIENTS WITH ISOLATES
100
80
60
40
20
BEFORE TREATMENT
AFTER TREATMENT
CONJUNCTIVAL SACS
LID MARGINS
ANTERIOR NARES
MORE THAN ONE SITE

Fig.VIII,4

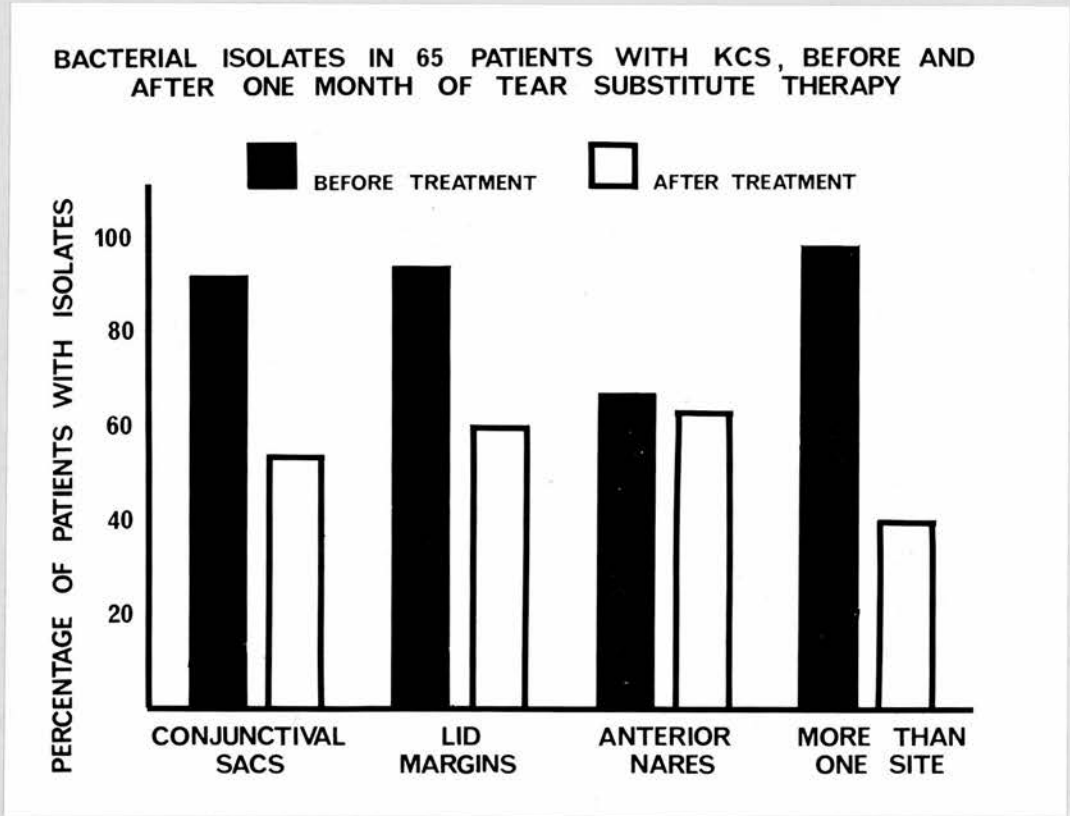
BACTERIAL ISOLATES IN 65 PATIENTS WITH KCS, BEFORE AND AFTER ONE MONTH OF TEAR SUBSTITUTE THERAPY
BEFORE TREATMENT
AFTER TREATMENT
PERCENTAGE OF PATIENTS WITH ISOLATES
100
80
60
40
20
CONJUNCTIVAL SACS
LID MARGINS
ANTERIOR NARES
MORE THAN ONE SITE

Fig.VIII,3

group of patients suffering from untreated keratoconjunctivitis sicca in that there are significantly more patients with staphylococcus aureus on the lid margins than in the nasal vestibule ($X^2 = 6.4243$, $P < 0.02$). However, the difference in sac isolates is less striking ($X^2 = 2.8053$, $P < 0.1$).

Staphylococcus aureus was isolated from more than one source in 17 of the 65 patients (26.1 per cent) suffering from untreated keratoconjunctivitis sicca, significantly more than the four patients of 60 (6.6 per cent) in the control series ($X^2 = 4.8442$, $P < 0.05$, Figure VIII,2). It is interesting to note that 13 of the 17 keratoconjunctivitis sicca patients were nasal carriers. Coagulase positive bacteria were present in the anterior nares of 16 patients and additionally, on the lid margins and conjunctival sacs of 6, on the lid margins of 4 and in the conjunctival sacs of 3. Another 4 patients had staphylococcus aureus in their sacs and lid margins but were not nasal carriers.

Tables VIII,7,8 and 9 show the frequency and identity of bacterial isolates from the conjunctival sacs, lid margins and anterior nares of 65 patients with keratoconjunctivitis sicca immediately following one month of tear substitute therapy. There was a significant reduction in sac ($X^2 = 3.5975$, $P > 0.05$) and lid margin isolates ($X^2 = 2.9770$, $P > 0.05$) - presumably as a result of the treatment (Figure VIII,3). However, although there was a lowered incidence of staphylococcus aureus in the conjunctival sac ($X^2 = 3.3497$,

$P > 0.05$), there was no difference in the lid margin isolates of this organism ($X^2 = 2.5260$). In other words, staphylococcus aureus was still to be found more frequently than in the control series (Figures VIII,2 and 4).

(b) Clinical evidence of infection was recorded in 37 of the 65 patients (56.9 per cent) in whom the diagnosis of keratoconjunctivitis sicca had been made (Table VIII,10). Blepharitis, occasionally accompanied by other signs suggesting possible bacterial infection, was diagnosed in 19 patients in the majority of whom staphylococcus aureus was detected, both in the conjunctival sacs (13 patients) and on the lid margins (16 patients). The prevalence of staphylococcus coagulase positive organisms is, therefore, higher in patients with superimposed blepharitis than in the keratoconjunctivitis sicca group as a whole but not significantly so.

Corneal lesions unassociated with objective evidence of blepharitis were recorded in 13 of the 37 patients with suspected clinical infection. Only four patients had staphylococcus aureus in their conjunctival sacs and 7 on their lid margins, isolation rates that are no higher than for the rest of the keratoconjunctivitis sicca group. Although the number of patients is too small for definite conclusions to be drawn, the results suggest that staphylococcus aureus is more likely to be associated with the blepharitis sometimes present in patients with keratoconjunctivitis sicca than with any

Coagulase positive Staphylococci in untreated keratoconjunctivitis sicca, related to clinical evidence of infection.

	Before one month tear substitute therapy			After one month tear substitute therapy.			
Clinical group	No.patients	No.conj.Staph. aureus	No.lid margin Staph.aureus	No.nasal isolates	No.conj. Staph.aureus	No.lid margin Staph.aureus	No.nasal Staph.aureus
Blepharitis	19	13	16	4	7	12	5
folliculitis	2	1	2	1	1	2	1
styes	2	2	1	-	-	2	-
yellow conj. discharge	2	1	2	1	-	1	-
Marginal corneal infiltration	1	1	1	-	1	-	1
Oral, ragged ulcerations cornea	1	0	1	-	-	-	-
Corneal lesions	13	4	7	3	2	2	1
Marginal corneal infiltration	1	0	1	1	-	-	-
Superficial punctate erosions, oval or ragged ulceration	12	4	6	2	2	2	1
Styes	2	1	1	-	-	1	-
Yellow conjunctival discharge	3	2	1	1	1	-	1
Total	37	20	25	8	10	16	7

Table VIII,10

	Antibiotic resistance	29 Staph.aureus isolates in control group		83 Staph.aureus isolates in keratoconjunctivitis sicca group	
		Group Number	Per cent	Group Number	Per cent
1.	Penicillin	7	24	30	36
2.	Ampicillin	1	3	5	6
3.	Cloxacillin	-	-	-	-
4.	Tetracycline	-	-	-	-
5.	Chloromycetin	-	-	-	-
6.	Erythromycin	-	-	-	-

Table VIII,11

specific corneal lesions. On the other hand, the presence of blepharitis in these patients may enhance the chances of conjunctival and corneal infection.

Antibiotic Resistance

Resistance to penicillin was encountered in some of the staphylococcus aureus strains (Table VIII,11) but in none of the other species. Twenty four per cent of the staphylococcus aureus recovered from the control series and thirty six per cent from the untreated keratoconjunctivitis sicca group were penicillin resistant. Of the 16 nasal carriers of staphylococcus aureus who had keratoconjunctivitis sicca, 10 (62.6 per cent) had penicillin resistant strains. A few patients had ampicillin resistant forms but all the bacteria were sensitive to cloxacillin, tetracycline, chloromycetin and erythromycin (Table VIII,11).

Discussion

The most recent surveys of bacterial flora in the healthy conjunctival sac undertaken in various parts of the world have indicated that staphylococcus albus and diphtheroids (Coryne bacteria xeroses) are by far the commonest organisms encountered (Duke Elder, 1965). To date, the only large series that has been published in this country concerns 5000 eye patients admitted to

hospital for routine ocular surgery (Smith, 1954). Bacterial isolates were detected in 53 per cent of Smith's patients, staphylococci of all types being present in nearly 34 per cent and diphtheroids in 31 per cent of cases. In the present study, 60 rheumatoid arthritic outpatients with no evidence of external eye disease were examined for conjunctival bacterial flora. Isolates were present in 60 per cent of the patients, staphylococci being identified in 26.6 per cent and diphtheroids in 22.3 per cent. Furthermore, staphylococcus coagulase positive organisms (Staph. aureus) were isolated in nearly 8 per cent of the patients in Smith's series and in 10 per cent of the rheumatoid group. The identity of the remaining organisms in the two series is also similar, more varieties being present in the larger series. It would appear, therefore, that the rheumatoid series of patients with no evidence of external ocular inflammation can be used as a control for comparison with the age and sex matched patients suffering from untreated keratoconjunctivitis sicca.

During the preliminary stages of these investigations, it was observed that nearly one in three new cases of keratoconjunctivitis sicca was suffering from blepharitis. Consequently, it appeared important to have a knowledge of lid margin as well as conjunctival flora in this group and in the control series. Of the 65 patients ultimately diagnosed 19 (29.2 per cent) had blepharitis (Table VIII,7). There was no significant difference

between the frequency and identity of conjunctival and lid margin bacteria in the control series. Similarly, no difference existed between the isolates from sacs and lid margins of untreated keratoconjunctivitis sicca patients. However, the higher prevalence of bacteria both in the conjunctival sacs and on the lid margins of patients with untreated keratoconjunctivitis sicca and the predominance of staphylococcus aureus were undeniable (Figures VIII,1 and 2).

A dramatic reduction in the frequency of bacterial isolates from the keratoconjunctivitis sicca patients resulted from one month of tear substitute therapy. The eye drops prescribed contained 0.005 per cent chlorhexidene digluconate as a preservative and this has some bacteriostatic effect. However, the therapeutic regime included frequent irrigation with dilute salt water and the mechanical effect of this procedure in removing mucus and possibly bacteria may have been considerable. Nevertheless, staphylococcus aureus was still more frequently isolated than in the control series (Figures VIII,2 and 4). Although the difference was no longer statistically significant, it appears to the author that a potentially dangerous situation continued to exist, particularly since it is well recognised that most strains of staphylococcus aureus in the human subject are virulent pathogens (Elik, 1959). The effect of corticosteroids in reducing resistance to a variety of bacterial and other infections is also well documented (Zimmerman, 1950;

Kligman, Baldridge, Rebell and Pillsbury, 1951; Selye, 1951; Symmers, 1965). However, some authors advocate the use of topical steroids in the treatment of the "dry eye" (Jones and Coop, 1965). The results of this study indicate that topical steroids should be used with extreme caution in the management of patients suffering from keratoconjunctivitis sicca.

Since the nose is generally regarded as the commonest site for staphylococcus aureus in man (Polakoff and colleagues, 1967) the nasal carrier states of the control series and the keratoconjunctivitis sicca patients were investigated. There was no difference in the nasal bacterial flora of the two groups of patients and staphylococcus aureus was more commonly detected in the anterior nares of the control patients than on their lid margins or conjunctival sacs. However, staphylococcus aureus was more frequently detected from the conjunctival sacs and lid margins of the untreated keratoconjunctivitis sicca group than from the nose (statistically significant in the case of lid margin isolates, Figure VIII,2, $X^2 = 6.4243$, $P < 0.02$). Moreover, significantly more patients suffering from untreated keratoconjunctivitis sicca exhibited staphylococcus aureus from more than one site ($X^2 = 4.8442$, $P < 0.05$). It is interesting to note that most of the patients affected were nasal carriers (13 of 17) and conversely most of the nasal carriers were simultaneously colonised by staphylococcus aureus around the eye (13 of 16). This suggests that the eyes of patients with untreated or inadequately

supervised keratoconjunctivitis sicca, are readily infected with staphylococcus aureus when the patient is a nasal carrier. Consequently, part of the management of keratoconjunctivitis sicca patients must entail the elimination of the nasal carrier state.

It was not possible to prove with certainty that the signs of clinical infection recorded in Table VIII,10 were the result of staphylococcal infection. Nevertheless, the prevalence of the staphylococcus aureus was higher in patients with blepharitis both before and after one month of tear substitute therapy than in any other group. Blepharitis, blepharoconjunctivitis, punctate keratitis, particularly of the lower half of the cornea, and marginal corneal infiltration, are all well recognised entities and all have been described in association with staphylococcal infection (Jones, Andrews, Henderson and Schofield, 1957; Thygeson and Kimura, 1963). All of these conditions were diagnosed in this study. However, staphylococcus aureus was also isolated from untreated keratoconjunctivitis sicca patients with no evidence of infection. It is possible to conclude that some signs of infection were not recorded as such but were interpreted as evidence of keratoconjunctivitis sicca. This criticism may well apply to "conjunctival injection" which the author regarded primarily as an indication of the dry eye.

It is beyond the scope of this investigation to study the reasons for variations in staphylococcal resistance to penicillin. Nevertheless, it is important

to record that in this area of the United Kingdom during the years 1965-68, 36 per cent of the staphylococci isolated from the external eye or the anterior nares of untreated keratoconjunctivitis sicca patients were resistant to penicillin. When any ocular infection develops in these patients, it seems logical to conclude that penicillin should not be prescribed, particularly if impairment of vision is likely to occur as a result of delay in appropriate treatment. None of the staphylococci were resistant to tetracycline or chloromycetin, therefore, these antibiotics should be used in the first instance.

Summary

A comparison was made of the bacterial flora of the lid margins, conjunctival sacs and anterior nares of 65 newly diagnosed cases of keratoconjunctivitis sicca and 60 age and sex matched rheumatoid arthritic patients with no evidence of external eye disease. The results, which took three years to collect, showed that significantly more bacterial organisms, staphylococcus aureus in particular, were isolated from the lid margins and sacs but not from the noses of patients suffering from untreated keratoconjunctivitis sicca. One month of tear substitute therapy was sufficient to reduce the incidence of bacteria in the keratoconjunctivitis sicca group, almost to that of the

control series. Nevertheless, staphylococcus aureus was still more frequently isolated from the keratoconjunctivitis sicca patients than from the control patients. Since coagulase positive staphylococci are usually virulent pathogens, it is suggested that topical corticosteroids should be prescribed with extreme caution in this disease.

Generally, the nose is regarded as the commonest carrier site for staphylococcus aureus. However, prior to treatment with tear substitutes, more staphylococci were found on the lid margins and sacs than on the noses of the keratoconjunctivitis sicca group. Moreover, most of the nasal carriers in the keratoconjunctivitis sicca group were colonised by staphylococcus aureus on the lid margins, the conjunctival sacs or both, which suggests that the nose may have been the origin of the infection. Therefore, attempts should be made to eliminate nasal carrier states in patients suffering from keratoconjunctivitis sicca. 37 of the 65 keratoconjunctivitis sicca patients (56.9 per cent) had clinical signs suggesting infection. Staphylococcus aureus was no more readily isolated from this group than from the keratoccnjunctivitis sicca group, as a whole, or the group of keratoconjunctivitis sicca patients without clinical evidence of infection. However, nearly 30 per cent had blepharitis and the prevalence staphylococcus aureus in these patients was higher before and after one month of tear substitute therapy

than in any other group. In addition, the variety of corneal lesions which were recorded have all been associated with staphylococcal infection in series, published by other authors. Thirty six per cent of the coagulase positive staphylococci were resistant to penicillin. None were resistant to chloromycetin or tetracycline, which are therefore recommended as the antibiotics of first choice.

STUDIES OF THE FUNGAL FLORA IN KERATOCONJUNCTIVITIS SICCA

Although the role of fungi as ocular pathogens has become more widely recognised (Maddren, 1941; Sykes, 1946; Mendleblatt, 1953; Mitsui and Hanabusa, 1955; Roberts, 1957; Haggerty et al, 1958; Mikami, 1958; Fine and Zimmermann, 1959; Chick et al, 1962; Currie, 1963; Ainley and Smith, 1965; Jelenkiewicz, 1965; Dominiczak et al, 1965), relatively little attention has been given to the fungal flora of the healthy or diseased eye. Previous lengthy investigations conducted in Europe (Fazakas, 1935, 1953) and in the U.S.A. (Hammeke and Ellis, 1960) have shown some disparity in the frequency of occurrence of fungi in the healthy conjunctival sac, and studies of smaller groups of patients (Mitsui and Hanabusa, 1955; Azevedo, 1962) may not have been representative due to the small size and selectivity of the samples. Therefore, before attempting to demonstrate a high incidence of fungi in the conjunctival sacs of patients suffering from keratoconjunctivitis sicca, the results from an investigation of clinically healthy conjunctivae of a large and representative group of subjects of various ages had to be analysed.

It is well documented that steroids reduce tissue resistance to a wide variety of bacterial, viral and fungal agents (Zimmermann, 1950; Kligman, 1951; Selye, 1951), and it would appear that the increase of ocular mycoses within recent years may be associated

with the extensive use of systemic and topical corticosteroids and of broad-spectrum antibiotics (Hogan et al, 1954; Suie et al, 1963; McLean, 1963). A number of patients with Sjøgren's syndrome are treated with systemic steroids and topical steroid therapy is advocated by some authorities dealing with the ocular manifestations of the disease. Consequently an investigation into the effects of topical steroids, topical steroids and antibiotics and systemic steroids on the conjunctival fungal flora of various groups of patients was undertaken.

Materials and Methods

1 Clinically Healthy Conjunctivae

553 patients (1106 eyes), comprising 284 males and 269 females, without clinical evidence of external ocular inflammation, were chosen for the investigation of the fungal flora of the healthy conjunctiva. Each decade was represented by at least 40 patients, equally divided between the sexes (Table VIII,12). The children in the 0-9 year age group were obtained mainly from an orthoptic department, and the 10-19 year olds from a large comprehensive school. The remaining subjects were drawn from an ophthalmic outpatient department and from a mass radiography centre.

The association of conjunctival fungi with topical

PATIENTS WITH CLINICALLY NORMAL CONJUNCTIVAE, BY AGE GROUP AND SEX.

Age Group (yrs)		0-9	10-19	20-29	30-39	40-49	50-59	60-69	70+	Total
Sex	Male	39	105	21	20	27	28	34	20	284
	Female	28	104	23	22	22	25	23	22	269
Total Patients		57	209	44	42	49	53	57	42	553

Table VIII,12 Healthy conjunctivae.

steroid therapy was examined in two series of patients.

2 Clinically Healthy Conjunctivae Subjected to Topical Therapy

Adult patients with no clinical evidence of external ocular disease selected from an ophthalmic outpatient department, and from the Centre for Rheumatic Diseases.

(a) Cultures were taken before and after a course of Betamethasone disodium phosphate (0.1% in water-miscible base) in 86 patients (26 males and 60 females; 165 eyes), applied three times daily for one week. The age range was 30 to 78 years (mean 62). 36 patients had various forms of cataract, 35 had refractive errors, and 15 had glaucoma.

(b) Cultures were taken from 52 patients (17 males and 35 females; 104 eyes) before and after a one week course of Betamethasone disodium phosphate 0.1% and neomycin sulphate B.P. (0.5%) in water-miscible base (Betnesol-N, Glaxo) three times daily for one week. The age range was 28 to 75 years (mean age 60.5 years). 25 of the patients in this group had various forms of cataract, three refractive errors, 23 rheumatoid arthritis and one systemic lupus erythematosus. Altogether 138 patients (269 eyes) with clinically healthy conjunctivae were thus "treated" with the

Patients Receiving Topical Steroid Therapy

OCULAR DISEASE.

Disease under Treatment	No. of Patients	No. of Eyes
Conjunctivitis	15	25
Blepharo-Conjunctivitis	10	20
Episcleritis	5	8
Anterior Uveitis	15	20
Scleritis	1	1
Total	46	74

Table VIII,13

DURATION OF TREATMENT

Duration	No. of Eyes
1-4 weeks	30
1-12 months	40
1-3 years	4
Total	74

Table VIII,14

topical steroid or with the steroid/antibiotic drops for one week, the fungal flora of the conjunctival sac being determined before and after the course of eye drops.

3 Diseased External Eyes Subjected to Topical Steroid Therapy

The second main clinical category comprised patients with various forms of external ocular disease, for which either betamethasone disodium phosphate or betamethasone disodium phosphate and neomycin sulphate had been prescribed.

(a) 46 patients (10 males and 36 females; 74 eyes; age range 15-58 years, mean 53) had been receiving the steroid preparations. Table VIII,13 shows the conditions being treated and Table VIII,14 the duration of steroid treatment. Mydriatics were being instilled into nine eyes, miotics into three and methylcellulose drops into one eye.

(b) 55 patients (10 males and 45 females; 75 eyes; age range 23 to 61, mean 54.5) had been receiving the steroid/antibiotic drops. The conditions under treatment are shown in Table VIII,15 and the duration of therapy in Table VIII,16. Mydriatics were being instilled into six of these eyes.

Patients Receiving Topical Steroid/Antibiotic Therapy

OCULAR DISEASE.

Disease under Treatment	No. of Patients	No. of Eyes
Conjunctivitis	16	21
Blepharo-Conjunctivitis	15	25
Episcleritis	1	1
Keratitis	15	19
Anterior Uveitis	4	4
Styes	4	5
Total	55	75

Table VIII,15

DURATION OF TREATMENT.

Duration	No. of Eyes
1-4 weeks	30
1-12 months	38
1-3 years	7
Total	75

Table VIII,16

4 Conjunctivae of Patients Receiving Systemic Steroid Therapy

The association of conjunctival fungi and systemic steroid therapy was examined in 30 randomly chosen ward patients who were being treated in the Centre for Rheumatic Diseases.

There were 8 males and 22 females; age range 18 to 57 years (mean 52). Two of the patients had recurrence of rheumatic fever, the remainder had rheumatoid arthritis. The duration of therapy varied from one month to more than 3 years and the daily prednisolone intake from 5 to more than 15 mgm. The total dosage of steroid ranged from 1.5 to over 99.0 G.

5 Conjunctivae of Patients with Sjøgren's Syndrome

Finally I was in a position to investigate and evaluate the fungal flora of 37 patients suffering from keratoconjunctivitis sicca (3 males and 34 females; 74 eyes; age range 37 to 80 years, mean 55.2 years). By the time the fungal surveys were underway the group of Sjøgren patients had been receiving tear substitute therapy for varying periods of time. For 4 days before the specimens were taken, the patients were asked to discontinue this regime. It was felt that any lengthier stoppage would be unfair to the patients who had benefited so much by this time. Indeed many were loathe to co-operate in the investigation when they

realised that they were to receive no treatment for a few days. Positive cultures were obtained from 10 patients and 8 of them agreed to stop treatment for a further 4 days after which a repeat culture was taken.

Collection of Specimens

In all cases, specimens for culture were taken from both conjunctival sacs using a stiff nickel chrome wire loop with which the lower fornices were vigorously scraped. In the case of the patients with keratoconjunctivitis siccaany ropy discharge was also utilized for culture. Any cultures found to be positive were repeated. 200 blank tubes inoculated in the clinics under similar working conditions were submitted to the laboratory interspersed in irregular batches with the clinical samples. Cultures were also taken at irregular intervals from the mydriatics, miotics vital dyes and carboxymethylcellulose drops in current use at the ophthalmic department.

Cultivation of Yeasts and Fungi

The mycological growth medium employed throughout this investigation was 2% malt extract agar, containing 0.036% potassium tellurite as a bacterial inhibitor. Following inoculation and transmission to the laboratory all tubes (including the blanks) were incubated at 25°C and were examined for evidence of fungal growth at

intervals of 10 days. Negative cultures were reincubated, and no tube was discarded before the end of 4 weeks incubation.

The yeasts and yeast-like fungi were identified according to the taxonomic descriptions of Lodder and Kreger-Van RIJ, (1952). In particular, the specific identification of Candida species was based upon the results of sugar fermentation and carbohydrate assimilation tests (Lodder and Kreger-Van RIJ, 1952). The identity of Candida albicans was confirmed by the demonstration of characteristic germ tubes in human serum (as described by Taschdjian et al, 1960), and by the demonstration of chlamydospore production on corn meal agar slide cultures. The identification of the filamentous fungi was based upon their gross colonial appearances on malt agar, and upon the nature and arrangement of their spores on malt agar slide cultures. The technique of slide culture was as described by Riddell (1950). In order to reduce bias in this study, the bacteriologist was not informed of the sources of the cultures.

Results

Table VIII,17 shows the frequency and generic identity of fungal isolates from the healthy conjunctival sacs in the various age groups. The overall incidence of fungi was 2.9 per cent, the lowest incidence, 0.8 per

ANALYSIS OF POSITIVE FUNGAL CULTURES FROM HEALTHY CONJUNCTIVAE, BY AGE GROUP.

Age Group (yrs)		0-9	10-19	20-29	30-39	40-49	50-59	60-69	70+	Total
Penicillium	Sp.	-	-	1	1	3	1	1	-	7
Aspergillus	Sp.	-	1	-	-	-	3	-	-	4
Rhodotorula	Sp.	-	-	2	-	-	-	1	-	3
Scopulariopsis	Sp.	-	-	1	-	-	1	-	1	3
Candida	Sp.	-	2	-	-	-	-	1	-	3
Isaria	Sp.	1	-	-	-	-	-	1	1	3
Geotrichum	Sp.	-	-	-	-	-	-	3	-	3
Papulospora	Sp.	-	1	-	-	-	-	-	-	1
Gliocladium	Sp.	-	-	-	-	1	-	-	-	1
Hormodendron	Sp.	-	-	-	-	-	-	1	-	1
Saccharomyces	Sp.	-	1	-	-	-	-	-	-	1
Rhizopus	Sp.	-	1	-	-	-	-	-	-	1
Nigrospora	Sp.	-	-	-	-	-	1	-	-	1
Total	No.	1	6	4	1	4	6	8	2	32
	Per cent.	0.8	1.4	4.6	1.2	4.0	5.6	7.0	2.4	2.9

Table VIII,17

PATIENTS WITH CLINICALLY NORMAL EXTERNAL EYES GIVEN A TOPICAL STEROID OR STEROID/ANTIBIOTIC PREPARATION.

Treatment Group	No. of Patients	Culture			
		First		Second	
		No. Positive	Organism	No. Positive	Organism
Betamethasone Disodium Phosphate	86 (165 eyes)	$2+^{ve}$	Aspergillus Rhodotorula	3	Aspergillus Penicillium Saccharomyces
Betamethasone Disodium Phosphate/ Neomycin Sulphate	52 (104 eyes)	$1+^{ve}$	Candida albicans	11 (3 (3 (3 (1 (1	Candida albicans Penicillium Aspergillus Streptomyces Trichoderma

Table VIII,18

cent, being observed in the 0 to 9 year age group, and the highest (7 per cent) in the 60 to 69 year age group. Although there were more isolates from patients over than from those under the age of 40 years, a progressive increase of incidence of isolates with increasing age was not demonstrated.

A comparison of the fungal flora of clinically normal external eyes before and after the topical application of steroid/antibiotic preparations for one week is shown in Table VIII,18. No significant increase in the frequency of isolation of fungi from the eyes of 86 patients treated with betamethasone disodium phosphate was observed but the application of betamethasone disodium phosphate/neomycin sulphate preparation was associated, over the same period of time, with a significant increase in incidence of fungi in the conjunctival sacs of 52 patients (X^2 Yates' correction = 8.2174; $P < 0.01$). On the other hand (Table VIII,19) the conjunctival sacs of patients with external ocular disease being treated with the steroid or steroid/antibiotic preparation did not yield fungi any more frequently than the healthy sacs. There was no significant difference in the isolation rates between patients subjected to topical steroids alone and those subjected to topical steroids combined with an antibiotic. Furthermore, repeats of the initially positive fungal cultures were uniformly negative, suggesting that fungal contamination was transitory. Only one positive culture was obtained from the 30

PATIENTS WITH EXTERNAL OCULAR DISEASE RECEIVING A TOPICAL STEROID OR STEROID/ANTIBIOTIC PREPARATION.

Treatment Group	No. of Patients	Initial Culture		Repeat of Initially Positive Cultures
		No. Positive	Organism	
Betamethasone Disodium Phosphate	46 (74 eyes)	1	Penicillium	Negative
Betamethasone Disodium Phosphate/ Neomycin Sulphate	55 (75 eyes)	4	Penicillium Aspergillus Scopulariopsis Rhodotarula	Negative

Table VIII,19

PATIENTS WITH SJØGREN'S SYNDROME.

Treatment	No. of Patients	Initial Culture		First Repeat of Positive Cultures		Second Repeat of Positive Cultures
		No. Positive	Organism			
Systemic Steroids	14 (28 eyes)	4	Penicillium Rhodotorula Rhodotorula Candida albicans	Negative	3	Negative Aspergillus Rhodotorula/ Candida albicans Not Repeated
No Systemic Steroids	23 (46 eyes)	6	Stemphylium Rhodotorula Candida albicans Tropicalis Penicillium Candida albicans Hormiscium	Negative	4	Candida albicans Penicillium Candida albicans Parapsilosis Candida albicans Not Repeated

Table VIII,20

patients receiving systemic steroid therapy.

Table VIII,20 shows the rate of occurrence of fungi in the conjunctival sacs of the 37 patients with Sjøgren's syndrome after 4 days without their usual ocular toilet. Four isolates were obtained from the 14 patients (28 eyes) receiving systemic prednisolone and six isolates from the remaining 23 patients (46 eyes) - an overall incidence of 13.3 per cent. This is a significantly higher incidence than that obtained from the clinically healthy conjunctival sacs investigated above ($X^2 = 5.258$; $P < 0.05$ comparing with age and sex matched normals). Further specimens taken from those patients following resumption of their replacement therapy did not yield fungi. Eight of the 10 patients in this group who had previously harboured conjunctival fungi were sampled once again 4 days after ceasing ocular treatment. Seven fungal isolates were obtained, four of which were species of Candida.

Two of the 200 blank cultures yielded aerial contaminants.

Discussion

Previous surveys of the fungal flora of the healthy conjunctival sac have been undertaken by Fazakas (1935, 1955) in Central Europe, by Mitsui and Hanabusa (1955) in Japan, by Hammeke and Ellis (1960) in the United States, and by Azevedo (1962) in Brazil. Ainley and

Smith (1965) are, to date, the only British workers who have undertaken a study of the fungal flora of the clinically normal conjunctiva. The latter was a small series comprising only 43 patients with no signs of disease of the external eye. In comparison, the present investigation has utilized material from 1106 healthy eyes, and is the largest series so far reported in this country. Fazakas (1953) and Hammeke and Ellis (1960) conducted the two previously largest surveys, in Europe and the U.S.A. respectively. In a study of 993 healthy eyes, Fazakas obtained 253 positive fungal cultures (an incidence of 25.4%). Hammeke and Ellis investigated 520 healthy eyes of adults, children, and neonates: 10.3% of 416 adults, 5% of 52 children, and 0.1% of 52 infants gave positive fungal isolates. Ainley and Smith (1965) studied only 43 healthy eyes, and obtained 12 positives (27.4%). I have obtained 32 positive cultures from 1106 healthy eyes, a much lower incidence of 2.9%.

The clinical methods seem to be similar to those of other investigators, and the lower incidence in this series may partly be explained by the cultural techniques employed. Thus, Ainley and Smith (1965) used Sabouraud's broth with subculture after one week to Sabouraud's agar plates, and subsequent incubation at 25°C for up to 6 weeks. Malt extract agar slants for primary isolation, and an incubation period extending to four weeks at 25°C was the technique used in this study. Hammeke and Ellis (1960) also employed Sabouraud's

glucose agar, but the length of incubation of their cultures is not stated. In general, primary incubation in broth might be expected to yield a higher isolation rate than on solid medium.

Previous workers have indicated that variations in the frequency of certain fungi occur, between various geographical regions; e.g. Candida species (Urrets-Zavalia, Remonda and Rammacciotti, 1958) and Sporotrichum sp. (Gordon, 1947; McGrath and Singer, 1952). Despite a low overall incidence of fungi, the results presented in this survey are in good qualitative agreement with other investigators in other geographical areas with regard to the genera of fungi and yeasts most frequently found in the conjunctival sac. Thus, Aspergillus spp., Rhodotorula spp., Candida spp., and Penicillium spp. appear to be common inhabitants of the healthy external eye. (Fazakas, 1953; Mitsui and Hanabusa, 1955; Hammeke and Ellis, 1960; Ainley and Smith, 1965). These fungi collectively accounted for 54% of the total isolates in this series. Fazakas (1953) found that the majority of his isolates from healthy eyes were moulds, 28% of the isolates in his series of 993 eyes belonging to the Penicillium group. 22 per cent of the positive cultures in the present "healthy eye" group were Penicillium.

In contrast to the findings of Hammeke and Ellis (1960), who reported distinct differences in the frequency of positive fungal isolates from conjunctival sacs in different age groups, progressive increase of

incidence of fungi with increasing age, was not recorded in this study, although the overall incidence of fungi in the older-age groups was somewhat higher than in the very young age groups.

One may question the significance of the presence of fungi in the healthy conjunctival sac. In an attempt to provide a partial answer to this question, all of the 32 positive cultures were repeated within 4 weeks of their initial detection, but only 4 repeat positives were obtained. Furthermore, in none of these cases was the same species recovered. On the basis of these findings, it is considered that the fungi cultivable from healthy conjunctival sacs must be regarded as transitory contaminants rather than resident commensals. Although it would appear therefore that little significance should normally be attached to the presence of fungi in the healthy conjunctival sac, nevertheless it is important to have accurate knowledge of the fungal species most likely to be encountered there even temporarily. Otherwise the significance of those found in disease and in particular in keratoconjunctivitis sicca could not be interpreted. The role of fungi as pathogens in ocular infections is becoming more widely recognised, and although an increased awareness of the possibility of ocular mycoses may be partly responsible, there is now well-documented evidence of a real increase in the incidence of mycotic infection of the eye (Haggerty and Zimmermann, 1958; Mikami and Stemmerman, 1958; Fine and Zimmermann, 1959;

Chick and Conant, 1962), especially as a sequel to trauma or surgery of the eye (Fine and Zimmermann, 1959), where infection is believed to be exogenous in origin. In the latter instance conjunctival saprophytes might assume a pathogenic role in an "opportunistic" infection, as recently discussed in the more general sphere of microbial disease by Symmers (1965).

The effect of corticosteroids in reducing resistance to a variety of bacterial, fungal and viral infections is well recognised (Zimmermann, 1950; Kligman and others, 1951; Selye, 1951; Symmers, 1965), and it is believed that corticosteroids may permit fungi, normally regarded as harmless commensals, to behave as pathogens (Agarwal, Malik, Mohan and Khosla, 1963; Suie and Havener, 1963). There is evidence that the extensive systemic and topical use of corticosteroids and broad-spectrum antibiotics has largely contributed to the increase of ocular mycoses (Hogan and others, 1954; Suie and Havener, 1963; McLean, 1963; Manchester and Georg, 1959; Wolter, 1962; Currie, 1963). There is also clear experimental evidence for the enhancement of the effects of fungus infection by corticosteroids (Mankowski and Littleton, 1954; Ley, 1956; Hirose, Yoshioka, Abe, Kanemitsus and Kiya, 1957; Agarwal and others, 1963). Although such experimental conditions may have little counterpart in human ocular infections, there are frequent reports at the clinical level, of ocular mycoses complicating steroid therapy, especially in relation to the recognised pathogens of

the Candida species. Sykes (1946), Mendleblatt (1953), Mitsui and Hanabusa (1955) and Roberts (1957) have all reported corneal infections by Candida albicans. Maddren (1941) reported a case of severe angular conjunctivitis occurring in the course of extensive candidiasis in a woman, but Duke-Elder (1960) has stated that fungus infections of the conjunctiva are very rare. A case of ocular mycosis due to Candida parapsilosis was reported by Manchester and Georg (1959). Their patient was thought to have received corticosteroid and antibiotic drops for a long period before developing keratomycosis, the initial lesion being a superficial punctate keratitis. Currie (1963) described three cases of mycotic keratitis associated with corneal ulceration. Candida albicans was implicated, and he considered that steroids had aggravated the condition. Ainley and Smith (1965) have recently described a probable case of secondary keratomycosis due to Candida parapsilosis, which responded to the administration of Nystatin.

It is of interest that both Candida albicans and Candida parapsilosis were recovered in the isolates from the clinically normal conjunctivae in this series and that Candida albicans was represented in the isolates from patients receiving topical steroid/antibiotic preparations.

Previous studies have been undertaken of the effects of corticosteroid therapy upon the incidence of fungi in the eye. Mitsui and Hanabusa (1955) obtained 42

positive cultures from 62 patients receiving topical ocular steroids (67%), while a control group of untreated patients had an incidence of 18.5%. The majority of the isolates in their series were Penicillium, Candida, Saccharomyces, Aspergillus, or Rhodotorula spp. In a second experiment, these authors selected 18 cases, initially negative for fungi by smear or culture. Following topical application of hydrocortisone ointment for 3 weeks, the eyes of 9 of the subjects were fungus positive, Penicillium and Rhodotorula spp. predominating. Ainley and Smith (1965) failed to demonstrate any striking change in fungal flora following the application of a corticosteroid/antibiotic combination (Betnesol-N) to the eyes. Thus, of 15 patients initially showing negative cultures, only 3 became positive for fungi after the administration of drops or ointment thrice daily for not less than 2 weeks. They point out that the number of patients studied was too small to give a statistically significant result.

In the present investigation, topical betamethasone treatment of patients with clinically normal external eyes and of patients with external ocular disease, did not result in any significant changes in the mycotic flora of the eye. Thus, 86 patients (165 eyes) with clinically normal external eyes received betamethasone disodium phosphate three times daily for 1 week; only two pre-treatment isolates and three post-treatment isolates were obtained. Furthermore, no generally

accepted fungal pathogens were represented. 46 patients (74 eyes) with external ocular disease, who had been receiving topical betamethasone disodium phosphate for varying periods of time, yielded one fungal isolate, a saprophytic Penicillium species and a further conjunctival sac scraping from the same patient was negative.

Four fungal isolates, of no pathogenic significance, were obtained from the eyes of 55 patients (75 eyes) with various forms of external ocular inflammation, who had been receiving the betamethasone disodium phosphate/ neomycin sulphate preparation for varying periods: repeat cultures were uniformly negative. The combined steroid/antibiotic preparation, therefore, produced no significant changes in fungal flora in this group.

The effects of the topical administration of the betamethasone/neomycin preparation to the patients with clinically normal external eyes deserve comment. Thus, there was one isolate from 104 eyes before the commencement of therapy, and 11 isolates, including three strains of Candida albicans, following its completion. It is difficult to offer a simple explanation of the comparatively large increase of ocular fungi in this particular group of subjects, but it is of interest that almost 50% of the patients in this category were hospital inpatients (23 rheumatoid arthritis; 1 systemic lupus erythematosus). When the cultural results are considered in relation to the source of the patient, 10 of the 11 post-treatment isolates were derived from the 24 ward patients, whereas

only one post-treatment isolate was obtained from the remaining 18 outpatients. The higher incidence of fungi in the ward patients might possibly reflect a high level of aerial fungal contamination of the ward environment when the specimens were collected. On the other hand, only one fungal species was recovered from 30 inpatients with rheumatoid arthritis receiving systemic steroids in the same wards as the former group.

There is no evidence from the present investigation, that the neomycin component of the combined topical steroid/antibiotic preparation made any significant contribution to the alteration of fungal flora in patients so treated. Thus, there was no significant difference in the frequency of isolation of fungi from the eyes of patients with external ocular disease treated with steroid/antibiotic and steroid alone (Table VIII,12). It is, however, relevant to note that numerous workers have shown that antibiotics, especially the tetracyclines, can enhance the growth of fungi, notably Candida albicans, and that subjects treated with antibiotics are more often carriers of Candida albicans than are untreated controls (McGovern, Parrott, Emmons, Ross, Burke and Rice, 1953; Sharp, 1954). There are relatively few reports to incriminate neomycin in this respect, though Reiersol (1958) observed a marked increase in the incidence of faecal Candida albicans in patients given oral neomycin, and it is possible that local neomycin therapy might give rise to a similar situation in the conjunctiva.

The Sjøgren group of patients yielded some interesting results. 10 primary isolates were obtained from 74 eyes. The patients were instructed to use no local treatment, not even saline washouts, for 4 days prior to the first culture. Thereafter, they were allowed to return to their usual routine of twice daily irrigations with saline and instillations of carboxymethylcellulose drops (0.5% solution) at least four times daily. The second cultures were taken while the patients were receiving this irrigation regime, and the totally negative cultural results could be adequately explained on the basis of mechanical removal of foreign material from the conjunctival sacs by the irrigations. This thesis is supported by the fact that seven fungal isolates were subsequently obtained from the eyes of eight patients who consented to stop all local treatment for four days before the collection of further specimens. Any fungistatic effect of the carboxymethylcellulose drops (which contains chlorhexidine digluconate, 0.05%) was excluded by failure to demonstrate inhibition of 20 strains of Candida albicans, three strains of Rhodotorula, and two Aspergillus strains, in simple plate diffusion tests in agar.

The results indicate that the untreated dry eye of the Sjøgren patient is more susceptible to colonisation with fungi than the healthy eye. As already stated some patients suffering from Sjøgren's syndrome may be receiving systemic steroid therapy. This study shows

that the isolation rate of fungi from rheumatoid arthritic patients who do not have keratoconjunctivitis sicca is unaffected by systemic steroid therapy and similarly the isolation rate from Sjøgren patients is not influenced by systemic steroids. In view of the more frequent isolation of fungi from the Sjøgren patients, including the potential intra-ocular pathogens Candida albicans and Candida parapsilosis, it is concluded that a constant watch must be kept on these patients' eyes for evidence of mycotic infection. Finally, particular caution must be exercised prior to any surgical procedures since it is known that fungi may be introduced as a result of trauma (Fine and Zimmermann, 1959).

Summary

Studies of the fungal flora of healthy and diseased conjunctival sacs had to be undertaken before any reliable comments could be passed on the incidence of fungi in keratoconjunctivitis sicca.

Fungi were isolated from 2.9 per cent of 1106 healthy conjunctival sacs, a higher incidence being observed in older age groups. Although the majority of the species isolated were non-pathogenic transient aerial contaminants, some potential intra-ocular pathogens were also represented.

Because topical steroids are advocated for the

treatment of keratoconjunctivitis sicca, the effects of topical administration of betamethasone and of a combined betamethasone/neomycin preparation of patients with normal and diseased external ocular tissues was observed. Topical betamethasone therapy did not result in any significant changes in the fungal flora of healthy eyes but a higher incidence was observed in the eyes of hospital inpatients treated with betamethasone/neomycin preparation. The outpatients who had external ocular disease under treatment with steroid or steroid and antibiotic applications had no more fungal colonisation than the healthy subjects.

Fungi were obtained from 13.3 per cent of the 37 patients suffering from Sjøgren's syndrome. This is significantly higher than matched healthy eyes. Whether the patients were receiving systemic steroids or not made no difference. The isolates included potential intra-ocular pathogens, Candida albicans and Candida parapsilosis and the untreated dry eye may be more susceptible to fungal colonisation than the eye receiving simple "replacement" therapy.

STUDIES OF THE VIRAL FLORA IN KERATOCONJUNCTIVITIS SICCA

Thirty per cent of 40 newly diagnosed untreated keratoconjunctivitis sicca patients had various corneal staining patterns when fluoresceine was stilled (Chapter II, page 40). Some of the lesions bore features not unlike early herpes simplex or adenovirus infections. In addition, two patients, while receiving treatment, developed corneal ulceration that spread out from a dendritic pattern to involve two-thirds of the corneal surface and extend into the anterior third of the stroma (Figs. VIII, 5 and 6). Jones and Coop (1965) recognised simplex corneal ulceration in some of their keratoconjunctivitis sicca patients and reported that some of them were carriers of the virus. However, no analysis of virus infection in Sjøgren's syndrome has been recorded to date. Consequently, corneal cells from patients suffering from keratoconjunctivitis sicca were examined for evidence of herpes simplex and adenovirus infection.

Two methods of detecting viruses in the cornea are at present in use; tissue culture and immuno-fluorescence.

Fig.VIII,5 showing spreading corneal ulceration that began in a dendritic pattern. Large mucous shred at 7 o'clock. Photograph taken immediately after instillation of B.J.6, thus highlighting the edges of the ulcer - patient No.20, appendix VIII.

Fig. VIII, 6 Dendritiform pattern of corneal ulceration photographed after instillation of B.J.6 - patient No.18, appendix VIII.

Patients

Between 1967 and 1968, 30 patients suffering from Sjøgren's syndrome and exhibiting fluorescein staining of the cornea were examined for evidence of adenovirus, herpes simplex and TRIC viruses by immunofluorescence techniques.

The investigations for corneal viruses were repeated in 8 patients and in a further 20 cases during 1969, using standard tissue culture methods for herpes simplex and adenoviruses.

Collection of Specimens, Techniques

Immunofluorescence

Suspect corneal epithelial cells were removed with a flattened aluminium spatula after instillation of a one per cent solution of amethocaine. The cells were spread over a clear glass slide, dried at room temperature, fixed in 90 per cent acetone for 90 seconds and transported immediately to the laboratory.

According to the methods in current use at the Department of Virology, Belvidere Hospital, Glasgow, a polyvalent serum was developed in rabbits to herpes simplex, adenoviruses 3,7,8 and 14 and the TRIC group. This anti-serum was conjugated with fluorescein, thereby rendering any reaction with appropriate virus

antigen contained in the corneal cells visible under ultra-violet light.

Tissue Culture

Specimens of corneal cells were obtained by scraping the corneal surface with sterile cotton tipped sticks. The ends of the sticks were broken off and immersed in screw topped bottles containing tissue culture medium of HeLa cells. The specimens were transported immediately to the University Department of Virology, Gardiner Institute, Glasgow, where they were incubated for up to four weeks and examined by the methods in current use in the department.

Results

No viruses were detected by either method.

Discussion

This study has failed to show any evidence of herpes simplex, adenoviruses or TRIC viruses in the corneal cells of patients suffering from Sjøgren's syndrome.

The procedure for obtaining corneal specimens for tissue culture is well established and gives a 90 per

cent isolation rate for herpes simplex from dendritic ulcers diagnosed at the University Department of Ophthalmology, Glasgow. Figures VIII,5 and 6 are certainly dendritic-like ulcers and it is difficult to explain negative results in these cases. It is possible that the patients had a form of corneal ulceration akin to spontaneous corneal perforation. However, reduced corneal sensitivity was not recorded in either patient.

The collection of specimens in the immunofluoresceine study was also similar to that used in other series. However, Richardson and his colleagues (Richardson, Crombie, Gardner and McQuillin, 1969) deposited the corneal cells in phosphate buffered saline and fixed them in acetone for 10 minutes at $4^{o}C$. An examination of the value of immunofluorescence in diagnosing virus infections is beyond the scope of this thesis. It is sufficient to record at present that no viruses were detected by this technique or by tissue culture in patients suffering from Sjøgren's syndrome.

Summary

Some of the staining patterns in Sjøgren's syndrome suggest early herpes simplex or adenovirus infections. For this reason corneal cells of 30 patients were examined by immunofluorescence methods and those of 28

patients by tissue culture on HeLa cells. Eight patients were examined by both techniques.

No viruses were detected.

SUMMARY

Although it has been stated in the literature that conjunctival and corneal infection is common in K.C.S., there do not appear to be any publications describing the prevalence of this complication in a series of patients. This Chapter is a careful documentation and investigation of ocular infection in K.C.S. The conclusion of these studies is that bacterial infection and fungal colonisation is significantly high in untreated K.C.S. but that it is greatly reduced by lubricant therapy. A controlled study of combined antibiotic/corticosteroid topical therapy revealed a high fungal infection complication rate. Penicillin resistant staph. aureus particularly in the presence of blepharitis are the most prevalent of the pathogenic bacteria. No viruses were detected.

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CHAPTER IX

THE TREATMENT OF KERATOCONJUNCTIVITIS SICCA

The prescription of "correct" treatment for keratoconjunctivitis sicca is only one facet of the management of this perplexing disease. Encouragement, explanation and confidence in the medical staff are at least as important as either topical or systemic drugs. Knowledge on the part of the administrator is essential if mishaps are to be avoided (Figure IX,11 perforation of cornea following intensive topical anaesthetics).

The most satisfactory way of supervising Sjøgren patients is to create "dry eye" clinics from which a close liaison between patient, ophthalmologist and physician can be developed. This is a record of the achievements and failures of such an enterprise which spanned the years 1965 to 1970.

Success in the management of the ocular manifestations of Sjøgren's syndrome has been claimed by many authors and a bewildering variety of treatments have been in fashion from time to time. Table IX,1 shows the types of local therapy and their exponents from 1924 onwards. It is noticeable that beneficial results were claimed by some authors for all of the treatments on the list (except subcutaneous Pilocarpine injections which produce sweating and salivation but no lacrimation, Schoninger, 1924). Initially, enthusiastic claims were made for each of the systemic drugs listed in Table IX,2. However, later reviews were either more cautious or completely condemned earlier forms of treatment. Manschott in 1961 concluded that most forms of systemic therapy, e.g. vitamins, thyroid extracts and oestrogens

LOCAL THERAPY	AUTHORS	SUCCESSFUL	UNSUCCESSFUL
Subcutaneous Pilocarpine	Schoninger (1925)		+
	Stock (1925)		+
	V Grósz (1936)		+
Moist Compresses	Stock (1925)		
Ringer's solution	Verhoeff (1925)	+	
	Hamilton (1943)	+	
Liq. Paraffin	Engelking (1928)		
	Ask and Berg (1936)	+	
	V. Grosz (1936)	+	
Fibrinolysin	Weve (1928)	+	
Radiation	Schall (1930)	+	
	Beetham (1935)		+
	V. Grosz (1936)		+
	Fried and Goldzeiher (1944)		+
	Thomson and Sadie (1956)	+	
Physiol. Saline	Wissman (1932)	+	
	Ruttgers (1938)		
Contact Lenses	Beetham (1935)		
	Löhlein (1936)	+	
	Lutman and Favata (1944)	+	
Sul. Fosin	Flodgren (1935)	+	
	Dalsgaard-Nielsen E, Dalsgaard-N ielsen T (1837)	+	
	Andersen (1939)	+	
Obliteration of Canaliculi	Beetham (1935)	+	
	Sjogren (1938)	+	
	Rucher (1938)	±	
	V. Grosz (1939)	+	
	Schmidt (1940)	+	
	Bruce (1941)	+	
	Gifford and Colleagues (1943)	+ / −	
	Fried and Goldzicker (1944)	±	
	MacLean (1945)	+	
	Holm, S. (1949)	+	
	Jones and Coop (1965)	+	

Local therapy	Authors	Successful	Unsuccessful
White of Egg	Grossge baver (1937)	+	
Mugin	Hollwich (1938)	+	
Sod. Salicylate	Meisner (1938)	+	
Gifford's drops	Gifford, Puntenney and Bellows (1943)	+	
	Malbrain and Arrechea (1943)	+	
	MacLean (1945)		
Spectacles with Reservoirs	MacLean (1945)	+	
	Flynn and Schulmeister (1967)	+	
Steroids	Bourne (1952)	+	
	Denko (1960)		+
	Shearn (1961)	+	
	Jones and Coop (1965)	+	
	Crompton (1968)		+
Barrie-Jones 6-Formula	Jones and Coop (1965)	+	
Acetyl-cysteine	Jones and Coop (1965)	+	
	Absolon and Brown (1968)	+	
Polyvinyl alcohol	Sabiston (1969)	+	

Table IX,1 Forms of topical therapy since 1924.

Vitamin A	Beetham (1935) Sjogren (1938) Stahel (1938) Anderson (1939) Bach (1939) Gifford and Others (1943) de Roeth (1945) Lutman and Favata (1946) Holm (1949) Allington (1950) Raffle (1950) Rothman, Block and Hauser (1951) Robert (1953) Christiansson (1954) Weill (1957)
Vitamen B	Franceschetti (1942) Lutman and Favata (1946) Godt fredsen (1947) Robert (1953) Suróa (1955)
Vitamen C	Robert (1953) Lyon (1956
Liver	Sjogren (1940) de Roeth (1945) Allington (1950) Behrman and Lee (1950)
Iodides	Beetham (1935) Allington (1950) Weill (1957)
Parasymathomimetics	Gifford and others (1943) Thonard (1956) Hatch and Boese (1955) Thomson and Eadie (1956) Weill (1957)
Cervical Sympathectomy	Critchley and Meadows, (1933) Leirich (1947) Weill (1957)
Ovarian Hormones	Isakolvitz (1928) Breinlich (1937) Vehagen (1943) Fried and Goldzieher (1944) Bruchner (1945) Smith and Smith (1950) Behrman & Lee (1950)
Thyroid	de Roeth (1945) Smith and Smith (1950) Denko (1960)
Steroids A.C.T.H.	Stephens (1950) Frenbel, Hellinga and Groen (1951) Sjogren (1951) Cadman and Robertson (1952) Sjogren and Erikson (1952) Erlich and Greenberg (1954) Gaulhofer (1954) Gurling, Bruce-Pearson and Pond (1954) Schaposnik, Bergna and Lohte (1955) Wigley and Egan (1955) Eadie and Thomson (1955) Thomson and Eadie (1956) Crompton (1968)
Implantations of Pituitary gland	Feher (1955)
Antimalarial Drugs	Heaton (1959)
Immunosuppressive drugs	Crompton (1968)

Table IX,2 Forms of systemic therapy since 1935.

were of no value because they did not strike at the root cause of the disease, and although systemic steroids might improve the well-being of the patient, there was rarely any demonstrable increase in tear secretion. However, when acute exacerbations are characterised by lacrimal and salivary gland enlargement or painful dacryoadenitis a rapid, although short lived, remission may follow treatment with corticosteroids or adrenocorticotrophic hormone (Frenkel, Hellings and Groen, 1951; Gurling, Pearson and Pond, 1951; Jones and Coop, 1965). Despite every effort, some patients suffering from keratoconjunctivitis sicca continue to suffer considerable discomfort, which prompted Duke-Elder and Leigh (1965) to express the somewhat gloomy view that "in the majority of such cases the ophthalmologist is reduced to the expedient of judicious but impotent expectancy".

One point that appears to be certain, from my perusal of the literature, is the wide variation in the clinical course of the disease. Hence the effectivity of any treatment should be examined only when it has been in use over a lengthy period of time. Furthermore, since there is no treatment at present that will eradicate the disease, all that can be achieved is relief of symptoms and perhaps a reduction in the rate of irreversible damage.

PATIENTS STUDIED

Ninety-eight patients were supervised for no less than three years. All were suffering from definite rheumatoid arthritis, 88 females, 10 males; mean age 58.4 years; range 32 - 74 years.

Seventy of the patients were cases diagnosed for the first time by the author, the remaining 28 were referred by ophthalmic colleagues from various parts of the West of Scotland.

METHOD OF ASSESSMENT

Progress was estimated in terms of change in the symptoms and slit lamp signs recorded in Chapter II (page 39) and constant watch was kept for signs of infection as described in Chapter VIII (page 160). When the studies of bacterial flora had been completed, on the newly diagnosed patients one month of chloromycetin drops four times daily and chloromycetin ointment nocte were prescribed. Thereafter, "tear substitute" therapy was instituted. Each of the 28 patients referred by ophthalmic colleagues had lid margin, sac and nasal cultures on first acquaintance and were then treated with one month of antibiotics as above.

METHODS OF TREATMENT

(a) Replacement therapy

(b) Conservation of tears

(c) Mucolytic agents

(d) Hygiene, antibiotics, steroids

(e) Management of complications

(f) Future developments

(a) Replacement Therapy

Many attempts have been made to replace normal tear secretions and evidently some success was met with in at least a proportion of cases (Table IX,1). Nevertheless, the failure rate was high enough to stimulate continued search for satisfactory local treatment. Jones and Coop (1965) noted that patients with keratoconjunctivitis sicca tolerated topical sulphacetamides and wondered if the alkalinity of the solutions was an important factor. After a three year study involving 50 patients they concluded that the solution now known as BJ6 with a pH of 8.45 was the most satisfactory one they had tried. They state that it was quite obvious the patients preferred this solution to one per cent methylcellulose or any other artificial tears such as Gifford's drops.

Composition of BJ6* tear substitute.

Substance	Per cent
Sodium chloride	0.6
Sodium bicarbonate	0.45
Sodium carboxymethylcellulose	0.5
Chlorhexidine	0.005
Water for injection to 100 pH	8.45

* Barrie Jones

Duration of treatment years	No. of patients completing treatment	Effect on symptoms			Patients receiving alternative treatment at end of time allotted.
		Improved	No change	Worse	
1	98	65	18	15	19
2	79	60	10	9	14
3	65	52	5	8	9

Table IX,3 B.J.6 therapy.

ıration of reatment years	No. of patients completing treatment	Effect on signs			Patients receiving alternative treatment at end of time allotted.
		Improved	No change	Worse	
1	98	58	24	16	19
2	79	51	18	10	14
3	65	45	10	10	9

Table IX,4 B.J.6 therapy.

Method

All patients were given BJ6 drops and instructed to use them no less than four times per day. In addition, the eyes were to be bathed night and morning with cotton wool swabs soaked in sterile water to which had been added half a teaspoonful of salt per pint of fluid. This simple remedy was recommended for the mechanical removal of any excess discharge.

Results

Sixty-five (62%) of the 98 patients completed three years of BJ6 therapy without receiving any other treatment (Tables IX,3 and 4) but only 44 (49%) had fewer symptoms and signs (Table IX,5 and Fig.IX,1).

Successful treatment can be analysed in three ways; improvement in symptoms (Table IX,3), in signs (Table IX,4) or in both (Table IX,5). More patients showed subjective than objective improvement but the difference in this series is not significant. At the end of each year of BJ6 therapy almost the same percentage of patients had required alternative or additional therapy, 19 of 98 (18%) in the first year, 14 of 79 (18%) in the second and 9 of 65 (14%) in the third (Tables IX,3 and 4). The mean percentage "loss" over three years was therefore 16.6 per cent per year. If this trend were to be continued all of the 98 patients would have

Duration of treatment years	No. of patients completing treatment	Effect on both Symptoms and Signs		
		Improved	No change	Worse
1	98	52	10	14
2	79	45	1	6
3	65	44	2	6

Table IX,5 B.J.6 therapy

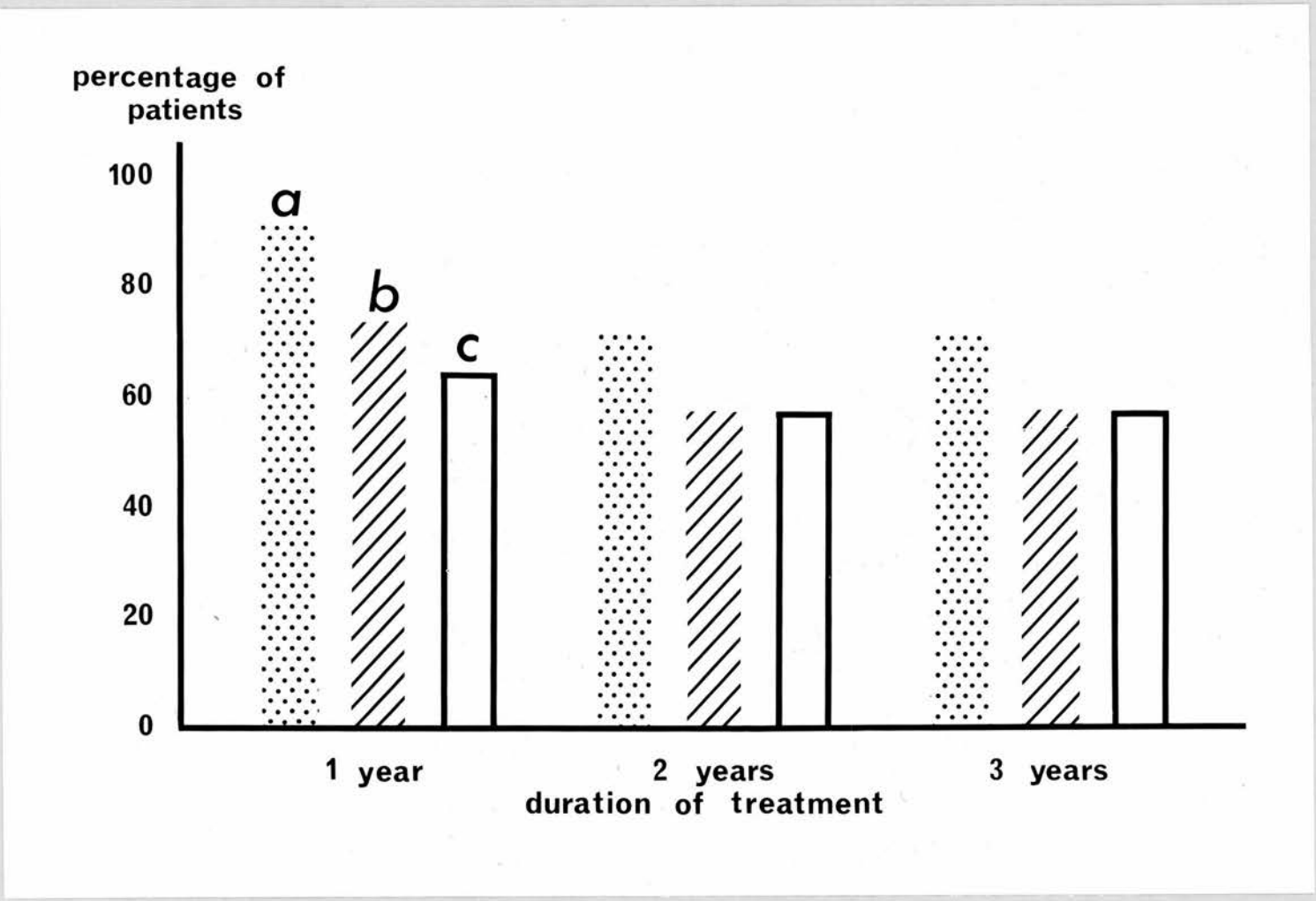

Fig.IX,1 Response to B.J.6 therapy; a = symptoms, b = signs, c = both.

required alternative or additional treatment within 14 years of first receiving BJ6 tear substitutes.

Reasons for changing or supplementing basic replacement therapy were increase in symptoms (5 patients), signs (9 patients), both (26 patients), recurrent lid infections (14 patients) and corneal lesions treated as suspected infections (7 patients). Of the 21 patients who showed evidence of infection, 16 were affected more than once. Nevertheless, BJ6 tear substitute was continued in these patients during and between courses of antibiotic therapy. 33 patients required naso-lacrimal duct occlusion and BJ6 was still useful in 10 cases after operation.

Discussion

Calculation of subjective improvement presents difficulties because so many factors are involved. The patient's personality, home commitments, weariness after a long ambulance journey and so on may colour the replies required to enquiries about symptoms. Nevertheless, some record of the patient's symptoms is helpful especially in a chronic disorder like Sjøgren's syndrome even if it only indicates a general trend. In this study more patients responded subjectively than objectively to BJ6 tear substitutes but nearly all of the cases whose signs diminished had symptomatic relief (Table IX,5). Sixteen per cent of patients per year

required alternative or additional treatment such as antibiotics, obliteration of naso-lacrimal canaliculi or mucolytic agents and it would appear that all of the 98 patients in this study will have required at least supplements to BJ6 therapy within 14 years. However, the carboxymethylcellulose solutions were still useful in a large number of patients who had required different treatment at some stage.

Summary

BJ6 therapy improved the symptoms and signs for three years in 49 per cent of 98 patients suffering from keratoconjunctivitis sicca. However, each year 16 per cent of the remaining patients required alternative or additional treatment and it is therefore unlikely that BJ6 alone can control the disease indefinitely. Nevertheless, patients benefit considerably from the instillation of this tear substitute and it is useful in conjunction with other remedies.

(b) Conservation of Tears

Naso-lacrimal Duct Occlusion

"Assuming that these irritable eyes are entirely the result of a lack of tears, we find ourselves considering the possibility of making better use of the tear supply that is available". (Beetham, 1935).

Beetham decided to destroy the naso-lacrimal ducts in four patients suffering from filamentary keratitis sicca with the idea in mind that the few tears being produced would be conserved in front of the eye for longer periods. In each case the improvement was immediate, pericorneal injection, corneal filaments and mucoid discharge disappeared and punctate staining areas were diminished. In addition, the measurable amount of tear secretion was increased. Beetham emphasised the need for diathermy of the canaliculi as well as the puncta to guarantee permanent occlusion. Since then many investigators have used this method and have met with varying degrees of success (Table IX,1). The main problem appears to be selection of suitable cases. Holm (1949) suggested that symptoms should have persisted for at least one year and that there should be heavy staining of both conjunctivae and corneae with rose bengal dye. Jones and Coop (1965) emphasised that patients must have visibly abnormal corneal epithelium and that occlusion should not be carried out during an acute exacerbation of any related connective tissue disease.

TABLE IX,6

Duration of treatment years	No. of patients completing treatment	Effect on Symptoms		
		Improved	No change	Worse
1	11	10	1	-
2	7	5	1	1
3	14	10	3	1
	32	25	5	2

Table IX,6 Obliteration of canaliculi and puncta.

TABLE IX,7

Duration of treatment years	No. of patients completing treatment	Effect on Signs		
		Improved	No change	Worse
1	11	8	3	-
2	7	4	2	1
3	14	8	5	1
	32	20	10	2

Table IX,7 Obliteration of canaliculi and puncta.

TABLE IX,8

Duration of treatment years	No. of patients completing treatment	Effect on both Symptoms and Signs		
		Improved	No change	Worse
1	11	7	-	-
2	7	4	1	1
3	14	8	3	1
	32	19	4	2

Table IX,8 Obliteration of canaliculi and puncta.

Method

Thirty two (34%) of the 98 patients suffering from Sjogren's syndrome required naso-lacrimal duct obliteration. Twenty five (27%) were selected because of increased symptoms and signs and 7 (15%) because of increased signs only. All of the patients had been supervised for at least one year and had received BJ6 during that time. In three patients the signs affected the conjunctivae only. The naso-lacrimal ducts and lacrimal puncta, upper and lower, were obliterated simultaneously by a diathermy current of 40 to 60 milliamperes. No fixed duration of application was used. When the skin overlying the canaliculus blanched the probe was slowly removed and the punctum sealed. Generally, 5 to 10 seconds was sufficient.

Results

The results are shown in Tables IX,6,7 and 8. A total of 25 of the 32 patients (76%) benefited from the procedure, 19 (57%) subjectively and objectively, 6 (18%) gaining symptomatic relief but no change in signs (Figures IX,2,3,4 and 5).

Eleven of the 32 patients (33%) were followed for one year, 7 (21%) for two years and 14 (42%) for three years. As in the previous study with BJ6 therapy, the number of patients gaining symptomatic relief was greater

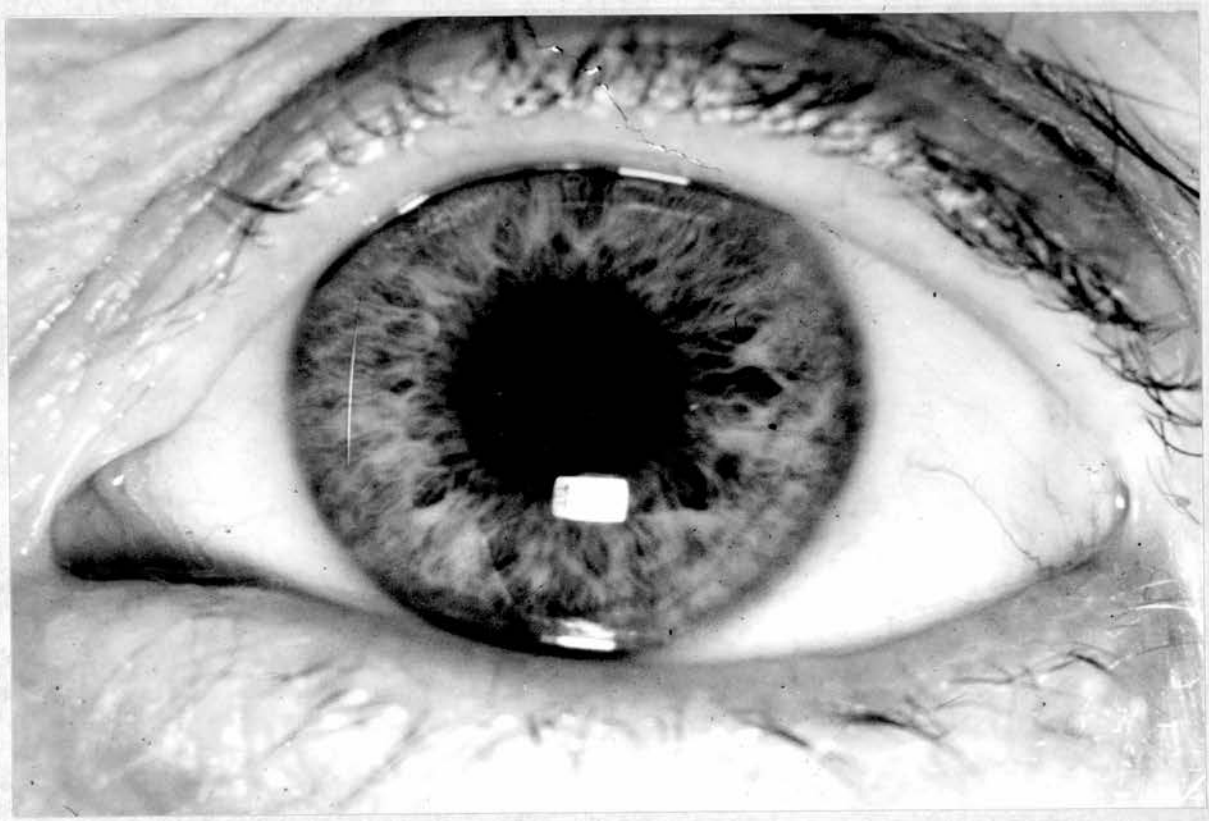

Fig.IX,2 Patient No.27, appendix IX, 3 years after N-L obliteration - no injection, normal moist eye.

Fig.IX,3. Patient No.17, appendix IX, 48 hours after N-L obliteration - slight injection still present - no mucous.

Fig.IX,4. Patient No.64, appendix IX.

Symptoms improved following N-L obliteration but signs still present. Site of previous corneal ulcer at 7 o'clock now quiescent. Folliculitis of lash follicles a recurrent problem with this patient.

than those showing objective improvement but the difference is not significant. About the same percentage of patients continued to show subjective and objective benefit with the passing of each year. Seven of 11 (63%) after one year, 4 of 7 (57%) after two years and 8 of 14 (57%) after three years (Figure IX,2). However, two patients continued on a downhill course despite obliteration of naso-lacrimal passages and BJ6 therapy (Table IX,6) and 8 patients were unaffected by the procedure. Ten of the 32 subjects (30%) continued with BJ6 after occlusion but were easier to control as a result of the surgery.

Discussion

The majority (76%) of the patients in this series who underwent naso-lacrimal canaliculus obliteration benefited from the procedure and there was no tendency for deterioration over a three years' period. Holm (1949) performed this operation on 23 cases. He observed the long term results in 14 patients finding that 8 (57%) were still much better, 6 of them free of trouble after 5 to 6 years. Although Holm was impressed by the relief of symptoms, he detected little difference in signs. In the majority of his cases filaments, mucous shreds and heavy rose bengal staining persisted. Only 6 patients (18%) in this series improved symptomatically without showing any change in signs.

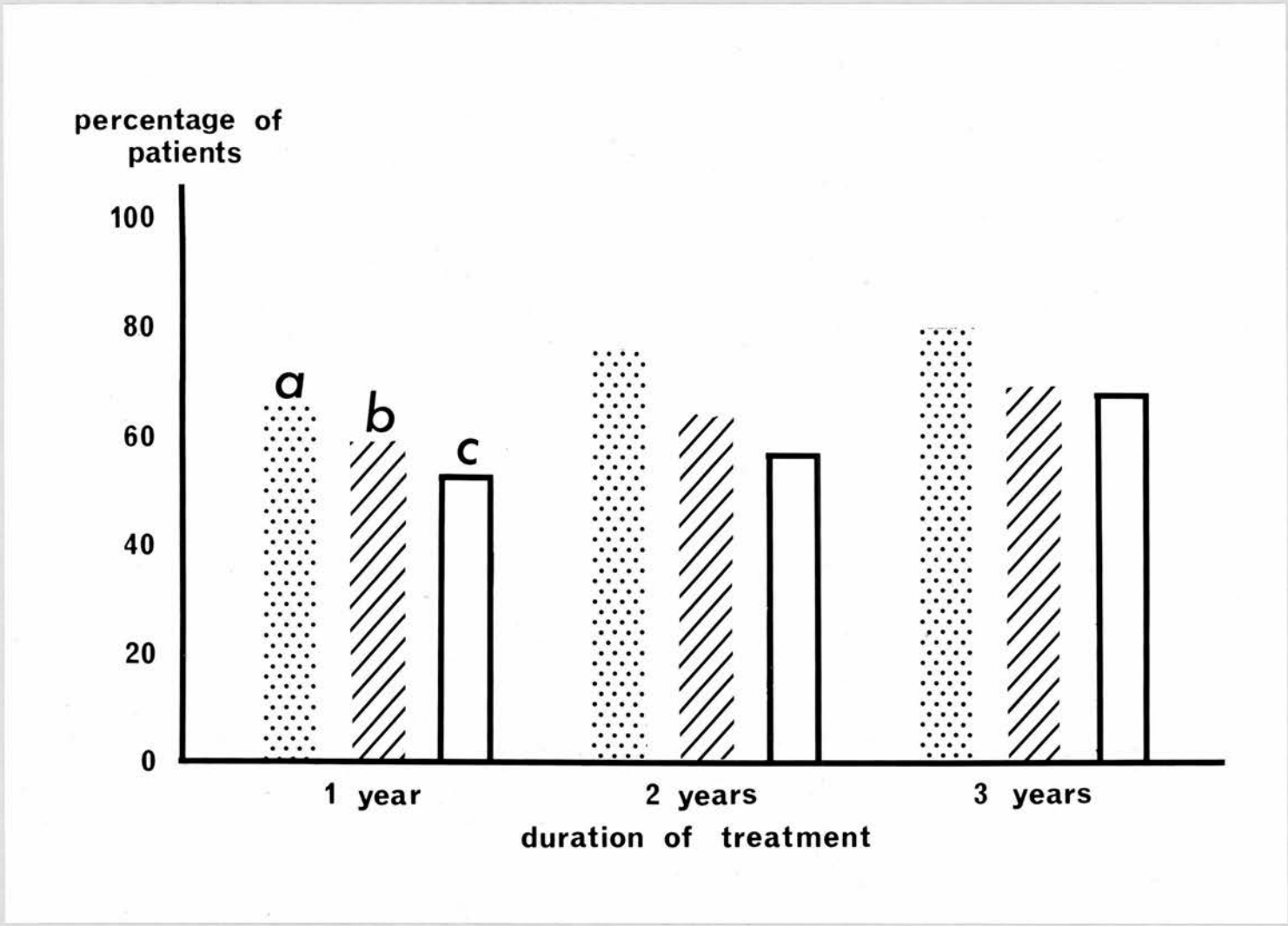

Fig.IX,5 Response to N-L duct and puncta occlusion; a = symptoms, b = signs, c = both.

Furthermore, Jones and Coop (1965) were of the opinion that provided abnormal corneal epithelium was present, occlusion of lacrimal outflow was always followed by improvement in both symptoms and signs. They emphasise the need to choose patients carefully. One of their failures had only slight corneal staining and had more trouble with the epiphora after operation than with his symptoms beforehand. In the present study, three patients with heavy conjunctival staining but no corneal activity were successfully treated and one of them has been followed for three years since the operation. There would appear to be three possible explanations for this apparent contradiction of Jones and Coop's criteria.

(1) Abnormal corneal epithelium was present in these three patients in between visits to the Clinics.
(2) The keratoconjunctivitis sicca was only saved from progressing to corneal involvement by the obliteration of the naso-lacrimal ducts.
(3) Abnormal corneal epithelium need not be the presiding factor influencing the decision for operation.

Mucous shreds and heavy conjunctival staining may be present in the absence of corneal filaments but in the presence of marked symptoms. In addition, Holm (1949) noted that even in patients who showed considerable symptomatic relief after occlusion of

lacrimal outflow, corneal filaments may persist. In this study 6 patients gained symptomatic relief without any change in their signs. These findings suggest that visibly abnormal corneal epithelium need not be the only criterion and that perhaps resistance of symptoms despite intensive topical therapy should be regarded as of prime importance in the decision to obliterate naso-lacrimal canaliculi and puncta.

Ten of the successfully treated patients were improved still further by the continued use of BJ6 tear substitutes. It is apparent that there is a need for more than one approach to the problem of treating keratoconjunctivitis sicca.

Summary

Thirty - two of the 98 patients with Sjøgren's syndrome did not respond to at least one year of BJ6 therapy and therefore underwent occlusion of the naso-lacrimal canaliculi and puncta. The majority of the 33 patients, 76 per cent, benefited from the procedure, 57 per cent both objectively and subjectively and improvement continued at the same level for up to three years. Visibly abnormal corneal epithelium need not be present in every case before operation is considered. Three patients in this group with normal epithelium benefited from occlusion of the lacrimal outflow. It is suggested that symptoms which resist current topical

therapy should be regarded as at least as important as the presence of corneal filaments.

Haptic Contact Lenses

Very few investigators have reported the result of treating keratoconjunctivitis sicca with contact lenses (Table IX,1). Only 3 patients in this group were fitted with haptic lenses and the results were uniformly bad. All three were severe examples of Sjøgren's syndrome who did not respond to BJ6 therapy or naso-lacrimal canaliculi occlusion. None of them was able to tolerate the lenses. Since simpler methods give relief to the majority of patients it would appear that the place for haptic lenses is limited, if there is one.

(c) Mucolytic Agents

It is very likely that excessive mucus is at least partly responsible for the corneal changes typical of keratoconjunctivitis sicca and corneal filaments seem to consist largely of mucin (Chapter II, page 42). Therefore, one aim in the treatment of keratoconjunctivitis sicca is the reduction of excess of mucus.

N-acetyl-L cysteine lowers viscosity by the reducing action of the free sulphydryl group in the molecule on the disulphide bonds of the muco-proteins in mucus. Its action finds widespread application in the treatment of respiratory tract disease (Webb, 1962). Jones and Coop (1965) reported encouraging short term results in the treatment of keratoconjunctivitis sicca with a 20 per cent solution of acetyl cysteine and Absolon and Brown (1968) in a double blind cross over trial lasting four months observed better results than with BJ6 tear substitute.

Method

Acetyl-cysteine reacts with rubber and begins to deteriorate after 48 hours exposure to air. Opened containers should be kept in a refrigerator at between 4 and 10°C (Stephens, Medical Information Department, British Drug Houses, 1968). In this study the drops

CHANGE IN SYMPTOMS FOLLOWING N-ACETYL-L CYSTEINE THERAPY

Duration of treatment months	No. of patients completing treatment	Effect on Symptoms		
		Improved	No change	Worse
1	20	8	11	1
2	20	10	9	1
3	19	11	8	-
4	19	9	10	-
5	19	8	11	-
6	19	8	11	-

Table IX,9 **Response to acetyl-cysteine.**

Duration of treatment months	No. of patients completing treatment	Effect on Signs		
		Improved	No change	Worse
1	20	10	9	1
2	20	10	9	1
3	19	10	9	-
4	19	8	11	-
5	19	7	12	-
6	19	7	12	-

Table IX,10 **Response to acetyl-cysteine.**

Duration of treatment (months)	No, of patients completing treatment	Effect on both Symptoms and Signs		
		Improved	No change	Worse
1	20	8	9	1
2	20	8	8	1
3	19	8	6	-
4	19	7	9	-
5	19	6	10	-
6	19	6	10	-

Table IX,11 Response to acetyl-cysteine.

were dispensed in plastic top bottles diluted to 5% solution with sodium bicarbonate added to a pH of 8.4. Patients were instructed to apply the drops at least four times per day, to keep the bottles in a refrigerator and to renew them after 48 hours.

Patients

Twenty patients who continued to display mucous shreds and corneal filaments after more than one year of BJ6 therapy were selected for treatment with 5% acetylcysteine. Eight of the patients had had their nasolacrimal ducts occluded without any beneficial effect. To comply with instructions from British Drug Houses, only those patients who had access to a refrigerator were included in the trial which was carried out over a period of 6 months.

Results

Results are shown in Tables IX,9,10 and 11, the same classification being adopted as in the previous studies. There was no difference in subjective and objective improvement throughout the 6 month period of the trial. One patient was withdrawn after two months of treatment because her symptoms and signs were worse. Her general health had also deteriorated, however, and secondary

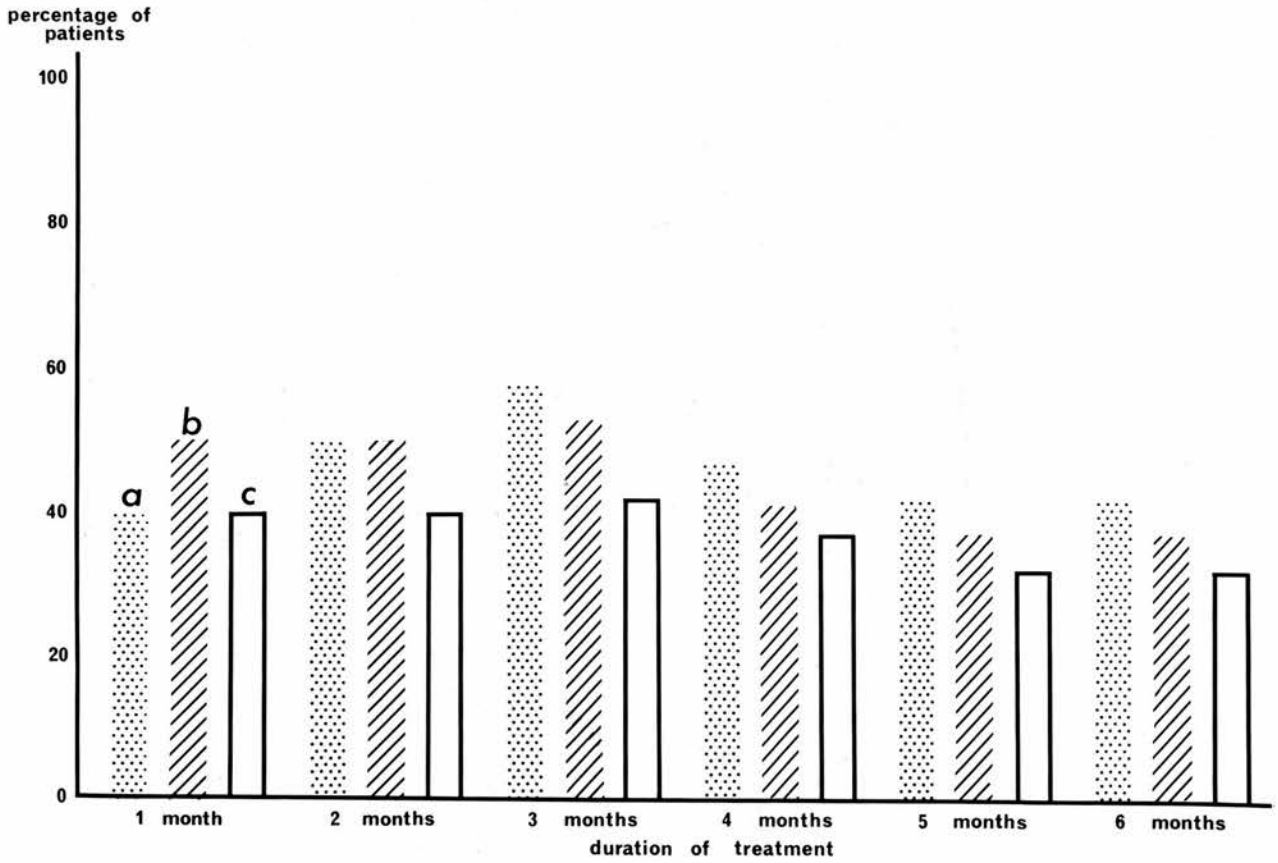

Fig.IX,6 Response to acetyl-cysteine; a = symptoms, b = signs, c = both.

infection was demonstrated. At the end of 6 months, 8 patients (40%) felt better, 7 (35%) had fewer signs and 6 (30%) had improved subjectively and objectively (Fig.IX,6).

Discussion

This small group of 20 patients was highly selected and therefore not truly representative of keratoconjunctivitis sicca patients as a whole. They had all failed to respond to at least one year of BJ6 therapy and 8 in addition had not benefited from occlusion of lacrimal outflow. Since acetyl-cysteine is a mucolytic agent, only patients who displayed corneal filaments and/or mucus threads were chosen for treatment. Therefore, this group of patients may have been worse examples of keratoconjunctivitis sicca than those reported by Jones and Coop in 1965 and Absolon and Brown in 1968. In addition selection in the present series was further reduced to those patients who had access to a refrigerator - a point not mentioned by either of the other two groups of investigators. All of the patients in the present group had definite keratoconjunctivitis sicca and rheumatoid arthritis (Sjøgren's syndrome). The associated diseases in Jones and Coop's group (1965) were not annotated and only 22 of the 30 patients in Absolon and Brown's study had rheumatoid arthritis.

Jones and Coop (1965) and Absolon and Brown (1968) prescribed 20 per cent acetyl-cysteine at a pH of 7. However, Stephens (British Drug Houses Ltd.) suggested that a 20 per cent solution might be irritating to the eyes and recommended dilution to 10 or 5 per cent. In this context, it is interesting to observe that Absolon and Brown (1968) thought some of their patients may have been unable to distinguish discomfort caused by the acetyl-cysteine from symptoms due to the disease. Furthermore, since mucus is dissolved more easily in an alkaline solution, the pH in this study was adjusted to 8.4. Over a period of 6 months, 30 per cent of the patients showed both objective and subjective improvement. Jones and Coop (1965) reported striking relief of symptoms in 13 of 15 patients (87%) with complete disappearance of mucus strands and ropy discharge but the duration of their observations is not stated. Absolon and Brown (1968) observed more improvement in signs in 30 patients treated for two months with acetyl-cysteine than with BJ6 but no difference in symptoms was reported.

The results of the present investigation are gratifying in that 30 per cent of 20 patients who were untouched by previous treatment reacted favourably to 5% acetyl-cysteine. Clearly, a longer trial is necessary and some method of preserving acetyl-cysteine for more than 48 hours would be of great benefit.

Summary

20 patients suffering from Sjøgren's syndrome who had received at least one year of BJ6 therapy without benefit were treated for 6 months with the mucolytic substance 5 per cent acetyl-cysteine in solution at a pH of 8.4. Eight of the group had also undergone naso-lacrimal duct and puncta obliteration without success and only those patients with corneal filaments and/or mucous threads were selected. Thirty per cent improved both subjectively and objectively and this was regarded as an encouraging result since the patients had been resistant to other forms of therapy. In the future a longer trial should be carried out and efforts should be made to find a suitable formula for stabilising acetyl-cysteine.

(d) Hygiene, Antibiotics and Topical Steroids

The prevalence of microbial agents in the dry eye as compared with the moist eye was examined in Chapter VIII. Evidently, the dry eye, especially the untreated one, is more susceptible to bacterial and fungal colonisation than the healthy eye. This being so the practice of strict personal hygiene must be a primary consideration in the management of keratoconjunctivitis sicca. The present group have been instructed to use separate face cloths and towels in an attempt to minimise infection from other members of their households. They have been warned of the dangers of rubbing their eyes with hands which may be unclean and have been advised against eye baths for the same reason. Inevitably, the catchment area for the patients studied in this series include sections of Glasgow not endowed with modern toilet facilities. In a few cases, the author has been able to initiate improvements in living conditions or even the provision of alternative accommodation.

Part of the regime of "replacement therapy" is twice daily bathing with dilute salt water prepared by the patient at home. When home conditions are inadequate and the patient's personal hygiene is in doubt, it is better to forgo this simple but effective supplement to the treatment of keratoconjunctivitis sicca.

Jones and Coop (1965) observe that staphylococcus aureus infections were so common in keratoconjunctivitis

sicca that there was "something to be said for the intermittent use of chloramphenicol ointment at night in many cases". In the previous Chapter (VIII) the prevalence of bacterial isolates, pathogenic staphylococcus in particular was shown to be higher on the lid margins and in the conjunctival sacs of untreated keratoconjunctivitis sicca patients than in a control series of rheumatoid arthritics. Overt blepharitis, sometimes accompanied by folliculitis, styes or yellow conjunctival discharge was present in about 30 per cent of newly diagnosed cases and the prevalence of staphylococcus aureus was highest of all in this group. Most of the pyogenic staphylococci were resistant to penicillin but not to chloromycetin or tetracycline.

All of the newly diagnosed patients and also those referred by ophthalmic colleagues were treated with chloromycetin drops four times per day and ointment nocte for the first month of their supervision. Repeat cultures were performed on the patients suffering from belpharitis and the antibiotics were continued until no bacteria could be detected. Nevertheless, 21 of the 98 patients (21.3%) developed evidence of infection during treatment with BJ6 tear substitute. Most of them suffered from belpharitis (14) the remainder from various corneal lesions and 16 of the group (16%) had recurrent bouts of infection requiring several courses of antibiotics (Fig.IX,7).

Topical steroids have never been prescribed for any

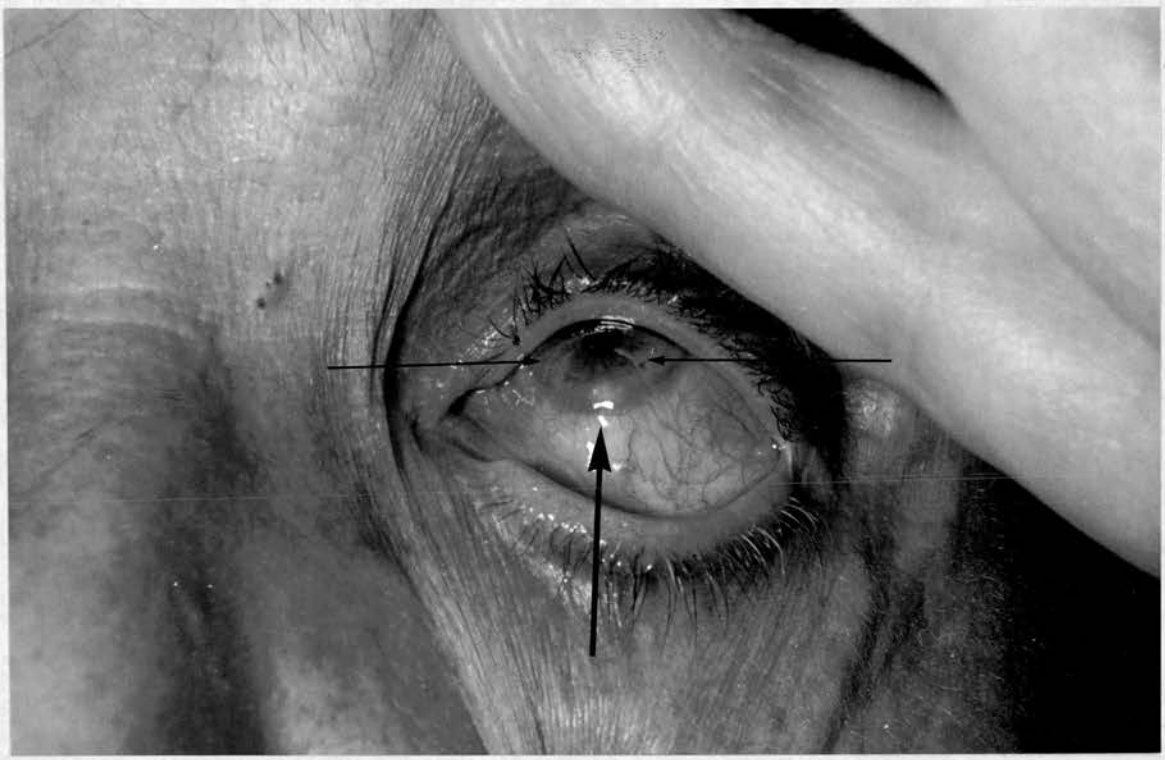

Fig.IX,7 Patient No.69, appendix IX.

Staph. pyogenes corneal ulcer photographed one week after commencing topical chloromycetin. Ulcer at 6 o'clock. Mucous shreds at 4 o'clock and 8 o'clock.

of the Sjøgren patients during the period of supervision under consideration. The prevalence of bacteria is so high and fungi colonise the untreated dry eye so readily that the author decided against corticosteroid therapy. One patient, a 45 year old rheumatoid arthritic of 10 years' duration had been using topical steroids for four years before referral to the clinic. She presented with bilateral steroid cataracts (Crews, 1963, Grade IV) and glaucoma as well as her keratoconjunctivitis sicca. Both cataracts had to be removed and drainage procedures performed. Thereafter, her ocular symptoms were quite adequately controlled with BJ6 tear substitute.

(e) Management of Complications

Serious corneal complications are unusual in Sjøgren's syndrome (Duke-Elder, 1965). Nevertheless, in this series 10 (10%) of the 98 patients suffering from keratoconjunctivitis sicca developed severe corneal lesions. The cases described in this section are by necessity highly selected in that none of them responded to at least one year of BJ6 therapy and obliteration of lacrimal outflow made no difference to their signs or symptoms. However, in other respects, age, duration and stage of arthritis, duration of ocular symptoms and laboratory data, they were no different from the rest of the keratoconjunctivitis sicca patients. Nor did the appearance of the corneal complications, in this study, coincide with an exacerbation of the patients' arthritis. The management of these patients and that of a further two cases is discussed under three headings, marginal corneal infiltration with vascularisation, perforation of the cornea and symblepharon with insufficient lid closure.

Marginal Corneal Infiltration with Vascularisation

Marginal corneal infiltration was diagnosed in six female patients. In two cases, the condition was present when they were referred by ophthalmic colleagues; the

others developed while under supervision at the "dry eye" clinics. In five instances, infiltration was monocular, in one binocular.

The anterior fibres of the stroma were the first to be involved generally at the lower third of the corneal margin. Gradual extension round the limbus and into middle stroma in some cases, was accompanied by vascularisation which was first evident as capillary loops lying behind the anterior edge of the infiltrated area. Three patients pursued a chronic course with slow advance in this pannus-like vascularisation. However, acute exacerbations characterised by disruption of Bowman's membrane and the overlying marginal epithelium were a recurrent feature of the others, one of whom also suffered two attacks of extensive corneal infiltration and anterior uveitis (Fig.IX,8).

Pathogenic staphylococci were isolated on three occasions from two of the patients who developed acute exacerbations but from none of the three whose disease followed a chronic course.

The significance of bacterial infection in this condition is difficult to judge. Chloromycetin was prescribed at the earliest sign of the corneal lesions. In retrospect, this action may have been unnecessary in the chronic cases who might have responded to topical steroids with a reduction in capillary formation. Beta radiation had no effect on one patient, but produced an impressive regression in the capillary loops in another,

Fig.IX,8 Patient No.74, appendix IX.

Repeated courses of topical antibiotics were necessary for this patient. Photograph taken one week after current course of neosporin had been started. Early pannus formation showing between 4 and 6 o'clock.

a beneficial result which has lasted for one year. Electrocautery applied to larger vessels had no lasting effect on a further two patients.

Perforation of the Cornea

Uniocular perforating corneal ulceration developed in three of the 98 patients under discussion (1 male and 2 female) and in a further case who was referred because of this complication. Corneal sensitivity was depressed in each case and ulceration began centrally with loss of epithelium and Bowman's membrane proceeding to stromal lysis and perforation within one to six weeks. One patient who had a three month history of ocular symptoms had received topical anaesthetics for six weeks and was referred as an emergency because of perforation of the cornea (Figure IX,11). The other three patients had been under supervision at the "dry eye" clinics; two had had occlusion of outflow and all three were in receipt of BJ6 therapy.

No bacterial organisms were isolated. However, a species of candida albicans was cultured from the cornea and genito-urinary tract of one patient who in addition had resistent staphylococci in a thigh wound following hip joint surgery (Figure IX,10).

The first case to present had a series of small central perforating ulcers of less than 0.5 mm. in diameter. She was treated conservatively with bed rest,

firm bandaging of the eye, topical chloromycetin and atropine four hourly, and systemic sodium fusidate (prescribed because of the high levels that this antibiotic can attain in human aqueous and vitreous, Williamson, Russell, Doig and Paterson, 1970). The corneal ulcers healed within a few days on three separate occasions; there have been no recurrences for the past 18 months.

The second patient, however, presented with a central corneal ulcer of 3 mm. diameter which had developed rapidly in the course of one week. The same treatment as in the previous case failed to prevent perforation which took place three days after admission to hospital. Iris prolapse, uveitis and secondary glaucoma resulted in a blind eye three weeks later (Fig.IX, 9). As a result of this disaster the methods adopted with future cases were less conservative.

The third patient was subject to recurrent guttate ulceration of the central cornea and had received several courses of antibiotics. She presented with a Descemtocele and was treated conservatively for three days. However, the cornea perforated and, with the loss of the anterior chamber, the lens came into contact with the posterior surface of the cornea. The next day after an ab externo approach the lens was extracted with a cryostat. The corneal perforation healed and the anterior chamber reformed within 48 hours. One year later there have been no further perforations and the patient has 6/18, N8 corrected vision (Fig.IX,10).

Fig. IX,9 Patient No.23, appendix IX.
Collapsed anterior segment following perforation of left corneal ulcer, uveitis and secondary glaucoma.

Fig.IX,10 Patient No.24, appendix IX, one year following extraction of lens. Eye white because of topical acetyl-cysteine and N-L occlusion but tendency to guttate ulceration at 6 o'clock still present. No further perforation.

The last patient was referred because perforation had developed following intensive topical anaesthetics. The diagnosis of keratoconjunctivitis sicca had not been suspected although the patient was known to be suffering from severe sero positive rheumatoid arthritis. Lysis of the ulcer with rapid extension to the limbus occurred two days after admission to hospital (Figure IX,11). Simple lens extraction was rejected because of the enormity of the perforation and a full thickness 10 mm. corneal graft incorporating a rim of sclera from 8 to 2 o'clock with extraction of the lens and complete iridectomy was performed (Figure IX,12). Systemic corticosteroids were prescribed in an effort to reduce the risk of rejection of the scleral rim (60 mgm. Prednisolone per day for 7 days, 50 mgm. per day 7 days, reduction by 5 mgm. per day to 20 mgm. maintenance dose for 8 weeks, 7.5 mgm. per day thereafter for six months). Unfortunately, iris presented through the wound which failed to heal in its lower third and two attempts at resuturing were only partly successful. Nevertheless, the graft maintained its clarity and three weeks after the original operation an amniotic membrane was placed across the entire anterior segment of the eye to protect the iris until vascularised tissue had grown over it. The amnion, however, was rejected within 24 hours. Nevertheless, at the fourth post-operative week vascularised conjunctival tissue began to grow over the prolapse. The patient was discharged from hospital

Fig.IX,11 Patient referred with perforating corneal ulcer, treated for 6 weeks with topical anaesthetic solutions. Iris adherent to posterior surface of corneal ulcer. Mucous shreds evident over lower third of cornea.

Fig.IX,12 Patient shown in Fig.IX,11 following corneo-scleral graft and lens extraction - small prolapse of iris at 5 o'clock.

Fig.IX,13 Central perforating ulcer in graft of patient shown in Figs.IX,11 and 12.

8 weeks after admission with a clear graft and a small temporal prolapse. Progress continued satisfactorily until the 14th post-operative week when a central ulcer developed in the graft and perforated within 48 hours (Fig.IX,13). Although the ulcer healed, bacterial infection could not be avoided and a panophthalmitis developed. The eye was removed 6 months after the graft operation.

Symblepharon with Insufficient Lid Closure

In advanced examples of Sjøgren's syndrome some degree of symblepharon may develop (Duke-Elder, 1965). The need for surgical intervention arose with only one patient in the series and in another referred as a case of essential shrinkage of the conjunctiva.

The first patient, a female of 73 years, had a history of symblepharon affecting the lower fornices of both sacs on two occasions during the preceding 6 years. The adhesions had been severed and were not present when the patient first came under care at the "dry eye" clinics. After three years of supervision the patient showed no improvement in signs and symptoms had increased. Symblepharon recurred in the right lower fornix and within 6 months of its appearance had resulted in defective lid closure. The adhesions were divided with round ended scissors and the fornix kept open by hourly glass rodding and chloromycetin ointment

for three days followed by four hourly treatment for one week. The patient refused systemic steroids because she had developed severe fluid retention 10 years previously following 6 months' of cortisone. There has been no further increase in the patient's symptoms or signs and no recurrence of the symblepharon over the past year.

The second patient, a male of 76 years, presented with essential shrinkage of the conjunctivae affecting the upper and lower fornices of both eyes (Fig.IX,14). He had a three year history of dimness of vision, recurrent episodes of pus discharge and a 10 year history that indicated the possibility of keratoconjunctivitis sicca (Chapter II, page 35). A diagnosis of Sjøgren's syndrome was made when examination revealed clinical and radiological evidence of rheumatoid arthritis. The eyelids were firmly adherent to the eyeball and were in a permanently open position three to four mm. wide. There was considerable pus discharge and the exposed corneal epithelium had sloughed. Ulceration extended into the deep stromal layers and pannus-like vascularisation encroached on the peri-central cornea especially in the left eye. A pocket of amniotic membrane containing a haptic corneal lens was inserted into the fornices of the left eye and secured with black silk sutures. The upper lid was stitched down to the cheek for four days during which time ocular movements were encouraged (Figures IX,15, 16 and 17). The contact lens was removed on the 12th post-operative day by which time most of the amnion had

Fig.IX,14 Essential shrinkage of conjunctivae in a 76 year old male rheumatoid arthritic with keratoconjunctivitis sicca. The eyelids on both sides are being forced as far apart as possible for the photograph. Slight movement in the right upper lid but none in the right lower lid is possible. The left eyelid movements are better following amniotic membrane grafts.

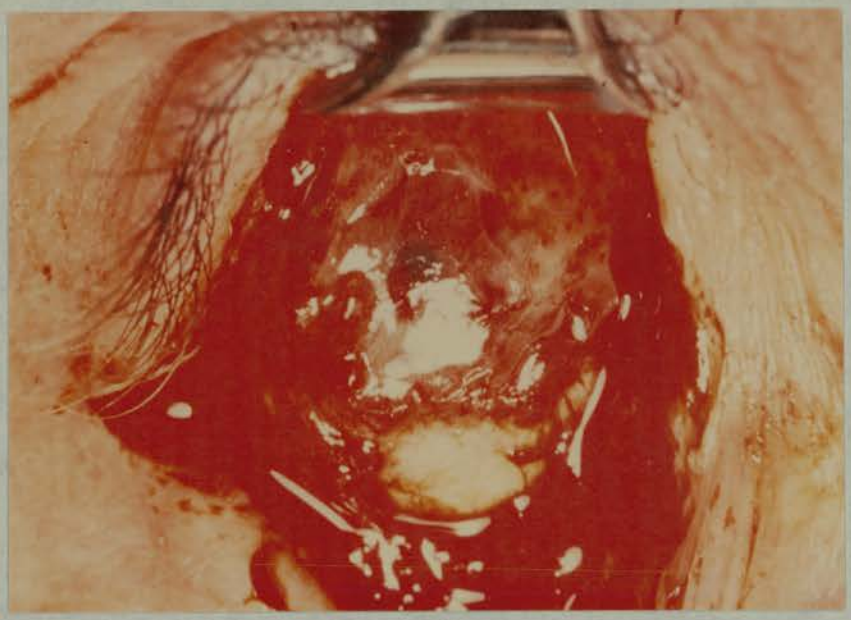

Fig.IX,15 Lids forced apart and dissected off the eyeball. Extensive pericorneal adhesions evident.

Fig.IX,16 Insertion of amniotic membrane.

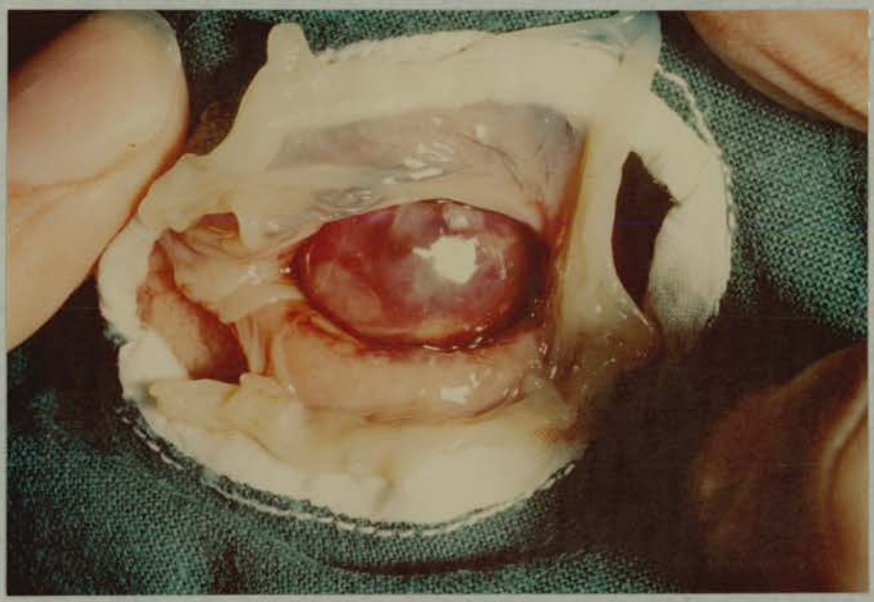

Fig.IX,17 Haptic corneal lens enclosed in pocket of amniotic membrane.

been discarded. The procedure was repeated in the right eye.

One year later, both eyes showed considerable improvement. The fornices were about half normal depth, lid closure though not complete was sufficient to protect the corneae and vision had improved to 6/36, N14 right eye, 6/60, N24 left eye.

Discussion

Ten per cent of the 98 patients in this series developed severe corneal lesions and a further two cases were referrod for treatment. The clinical and laboratory data of the patients who developed these complications were not significantly different from the rest of the keratoconjunctivitis sicca group.

Marginal corneal infiltration with vascularisation was diagnosed in six female patients, three of whom developed acute exacerbations associated with pathogenic staphylococci. Although response to antibiotics was dramatic in these three cases, pannus-like vascularisation continued to progress towards the centre of the cornea in all six. The anti-inflammatory effect of topical steroids was denied the patients because of the fear of infection (Chapter VIII). In retrospect, this approach may have been too cautious and in future steroids will be used in chronic phases and as a sequel to antibiotic therapy. Beta radiation caused regression in capillary

loops in one case but neither radiation nor electrocautery had any effect on vascularity of the cornea that had progressed beyond the capillary stage. It is proposed, therefore, that Beta radiation should be resorted to at the earliest sign of pannus formation.

The management of perforating corneal ulcers in Sjøgren's syndrome was not clarified in any way by the results in the four cases reported above. All four patients had reduced corneal sensitivity at the time the complications were diagnosed but it is difficult to see what can be done about this - if anything. Prophylactic tarsorrhaphy would appear to be a drastic procedure particularly since ulceration with perforation appears to be an inconstant complication of reduced sensitivity (Chapter II). Candida albicans was isolated from the eye and vulvae of one patient but examination for bacteria was negative in all cases. There does not appear to be any increased prevalence of infection, therefore, in patients suffering from this complication. Two patients developed small iris prolapses, the lens coming into contact with the posterior surface of the cornea around the edges of the perforation. The first was treated conservatively with disastrous results, the second responded well to lens extraction and abscission of iris prolapse. Consequently, it would appear that surgical intervention in these cases carries a better prognosis. On the other hand, another patient who developed small perforating ulcers without iris prolapse, responded well to firm bandaging and bed rest. The

remaining patient had been treated with topical anaesthetics for six weeks and presented with corneal perforation which extended rapidly towards the limbus necessitating a corneo-scleral graft with lens extraction. The immediate post-operative period was complicated by iris prolapse which resulted in defective wound healing. Perforation occurred in the graft three months after the operation and bacterial infection resulted in a blind eye. There are several factors that may have affected the outcome in this patient; topical anaesthetics, extent of the surgery required, iris prolapse and secondary infection. Clearly the only avoidable one was the prescription of topical anaesthetics.

Symblepharon requiring surgical intervention presented in two patients. The simple expedient of snipping the tissue with scissors and keeping the fornix free by glass rodding and ointment was quite sufficient in one case who had undergone the procedure on several occasions with results that lasted for several years at a time. Another patient responded satisfactorily to amniotic membrane grafts and haptic contact lenses. It would appear, however, that this extensive surgical approach could have been avoided if the symblepharon had been dealt with at an early stage.

Summary

Ten per cent of the 98 patients in this series developed severe corneal complications which were classified as marginal corneal infiltration with vascularisation, perforating corneal ulcers and symblepharon with insufficient lid closure.

Acute exacerbations associated with staphylococcus aureus infection characterised some of the cases with marginal infiltration. Nevertheless, it is advocated that a trial of topical steroids following antibiotic therapy might result in reduced vascularisation. Beta radiation resulted in an impressive regression of capillary loops in one patient but had no effect on larger blood vessels in two other cases.

Perforating corneal ulcers present a complicated problem. All of the patients in this group had depressed corneal sensitivity and excessive topical anaesthetic drops appeared to contribute to the extent of the perforation in one instance. Candida albicans was isolated from the cornea in one case but no bacteria were detected. Conservative management would appear to be sufficient in the presence of small perforations. However, in the event of iris prolapse with the lens brought into apposition with the edge of the ulcer, abscission of the protruding uveal tissue and lens extraction appear to give the best chances of recovery.

Symblepharon causing defective lid closure

responded satisfactorily in one patient to simple division with scissors. Extensive amniotic membrane grafts and haptic contact lenses produced an improvement in lid closure in a further case with severe shrinkage of the conjunctivae. It is suggested that earlier intervention in this case would have avoided the need for such radical surgery.

(f) Future Developments

The cause of keratoconjunctivitis sicca in Sjøgren's syndrome is, like that of rheumatoid arthritis, obscure. There are no known prophylactic measures and it would appear preventive therapy must await further elucidation of the whole disease process. For the moment, the ophthalmologist has to content himself with seeking better or at least alternative palliative treatment. In this context, it is noted that reduced lysozyme concentration in tears may precede all other evidence of keratoconjunctivitis sicca and is a constant feature of the established disease (Chapter II, page 44). A lysozyme solution designated MT-L ophthalmic has been used recently in the treatment of various inflammations of the external eye with good results (Matsushita, Tani and Miyaura, 1969). Consequently, a trial of this enzyme in keratoconjunctivitis sicca patients is being arranged at the time of writing.

In 1945, Maclean described glasses fitted with reservoirs containing fluid that could be conducted by means of a fine bore tube into the fornices of the wearer. He reported encouraging results with this apparatus in the management of keratoconjunctivitis sicca patients. Flynn and Schulmeister working in Australia (1967) recorded their experiences with similar models and came to the conclusion that further development was desirable. The concept of a constant

drip of fluid into the dessicated eye is certainly appealling, but to date satisfactory prototypes have not been developed in this country. At the present time the Western Regional Physics Laboratory is investigating the problem.

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A P P E N D I C E S

Abbreviations and Symbols in Sequence

Appendix II

* = Arthritic patients with false positive Schirmer I tests

1-250 Female patients

251-338 Male patients

K.C.S. = Keratoconjunctivitis Sicca

R.A. = Rheumatoid Arthritis

Appendix IV

L.T. = Latex test for rheumatoid factor

A.N.F. = Antinuclear factor

Glob. = Serum Globulin

Alb. = Serum Albumin

Alk. Phos. = Alkaline Phosphatase

Appendix VII

B = Biopsied thyroid gland

H = Hypothyroid not receiving treatment

Prec. Test = Precipitin test

T.R.C. test= Tanned red cell test

Fluor. 'C.F.' test = Fluorescent 'complement fixation' test

S.T. = Schirmer II test

Family History + of pernicious anaemia

O of thyroid disease

P.T.G. = Palpable thyroid gland

P.C.A. = Parietal cell antigen

T.A. = Thyroglobulin autoantibodies (all types)

Appendix VIII

S = Staph. aureus coagulase positive

C.I. = Clinical infection

Appendix IX

N-L = Naso-lacrimal puncta and canaliculi obliterations

Acet. Cyst. = Acetyl cysteine 5 per cent

Symptoms)	+	improved
)		
Signs)	0	no change
)		
Both)	-	worse

APPENDIX CHAPTER II

Effect of Temperature and Humidity

Age and Sex of Patients Studied

250 females; 88 males

	0	1	2	3	4	5	6	7	8	9
		46	54*	71	72	58	56	60	56*	61
1	64	67	57	34	25	18	18	27*	58	61
2	55*	52	55	65	59	59	68	68*	70	61
3	56*	54*	47	72	56	61	52	64	53	59
4	64*	51	37*	53	57*	60*	64*	58	49	72
5	52	59	54	58	68	56	61	61	57	72
6	33	53	52	72	68	71	63	68	56	58*
7	57	61	58	52	48	61	46	42	39	71
8	49	60	54	55	50	58*	57	48	38	68
9	64	62	63	71	18	25	35	38	44*	56
10	59	63	68*	61*	54	56*	56	57	51	49
11	69*	22	31	49	58*	59	70*	56	58	52
12	64	58*	63	68	67*	67*	67*	67	66	65
13	55	54	38*	44	64	62*	62*	62	63*	68
14	70	69	70	69	71	52	50*	50	49	50
15	51	58	59	61	62	63	56	51	50	48
16	47	61	71	61*	56	64	71	23	29	56
17	59	70	48	59	38	56	64	51	50	55
18	67	52*	54	57	62*	19	35	42	58	64
19	72*	55	57*	41*	57*	51	68	64	70*	48
20	56	55	31	63	62	51	59	57	62	47
21	58	57*	61	70	63	65	63	44*	70*	54
22	60*	63	48	56*	57*	49	44	55*	60*	51
23	50	60*	64	62	61	55*	46	60*	47*	38
24	62	70	70	62*	50	50	51	56	50	66
25	70	60	70	62	56	55	62	29	44*	62
26	61	57*	59	66	59	51	67	69	68	61*
27	61	58*	44	19	38	40	42	56	55	64
28	68	50	50	61*	62	51	50	61	64	71
29	25	38	42	49	50	52	52*	55	53	55*
30	49	54	41	58*	58	54	56	63*	51	53*
31	55*	46	44	56	57*	62	39	31	33	64
32	54	56*	58	61	61	70	55	30	34	41
33	71	64	65	63	62	57	56*	54	66	

Age Range 18-72 years. Mean = 55.47 years

The Keratoconjunctivitis Sicca Index for Physicians

The method of scoring is illustrated on page 250. The left hand column contains the numbers one to 17. Numbers one to 10 represent the 10 symptoms set out in Table II,2, numbers 11 to 17 the macroscopic signs set out in Table II,3 (following page 34 of the thesis). Each of the symptoms and signs was given a "loading value". For example, symptom one was valued at plus 5 points when present and minus one point when absent. The values for each of the 17 facts were evolved once the 40 newly diagnosed K.C.S. patients and 40 control patients had been examined.

The method was approved by Dr. John Anderson, Department of Biomathematics, University of Oxford.

AN ILLUSTRATIVE CASE

Bracketed numbers only are counted

	PRESENT	ABSENT
	+	-
1	(5)	-1
2	(5)	0
3	4	0
4	(5)	0
5	3	0
6	2	0
7	(3)	-2
8	2	0
9	1	0
10	2	0
11	2	0
12	2	0
13	(5)	-2
14	(3)	-1
15	(2)	0
16	2	0
17	(1)	0
TOTALS	30	0

TOTAL SCORE: 30

CASE NO.: 1 Female, M.S., aged 54 years.

DIAGNOSIS: Definite K.C.S.

Original K.C.S. Group of 40 Patients Index Score
1-32 female; 33-40 male

Patient No.	Age	Score
1	54	30
2	53	30
3	59	26
4	48	30
5	32	34
6	49	17
7	58	17
8	41	26
9	54	35
10	68	11
11	62	29
12	61	19
13	48	46
14	56	46
15	52	17
16	59	15
17	60	20
18	62	26
19	49	26
20	53	32
21	54	- 2
22	59	33
23	61	25
24	62	28
25	51	18
26	56	16
27	50	27
28	48	16
29	64	15
30	66	23

Patient No.	Age	Score
31	49	21
32	57	46
33	54	21
34	54	33
35	56	33
36	57	27
37	61	24
38	63	11
39	65	3
40	67	26

Mean Age	55.80 years
Range	32-68 years
Mean Score	24.4
Standard Error	4.23

Control Group of 40 Patients Index Score

1-28 female; 29-40 male

Patient No.	Age	Score
1	47	7
2	50	6
3	54	8
4	54	- 1
5	61	6
6	62	- 1
7	70	- 6
8	64	6
9	70	6
10	68	- 6
11	44	- 1
12	41	- 1
13	38	7
14	54	5
15	55	3
16	56	3
17	49	8
18	61	- 5
19	64	3
20	70	- 2
21	66	7
22	60	1
23	66	8
24	60	3
25	59	- 6
26	41	- 1
27	42	5
28	49	- 6
29	55	10
30	54	- 6

Patient No.	Age	Score
31	38	4
32	44	- 3
33	42	- 3
34	61	0
35	54	- 2
36	57	- 2
37	51	- 1
38	59	- 2
39	62	- 2
40	55	0

Mean Age		55.17 years
Range		38-70 years
Mean Score		1.18
Standard Error		4.48
Rule Score	$\leq$	10 normal
Score	$>$	10 K.C.S.

Scores in 100 R.A. patients to whom the K.C.S. Index was applied

(Case numbers adjusted for ease of recording)

Case	1 - 30	Score -6 to 0
Case	31 - 50	Score 0 to 4
Case	51 - 92	Score 4 to 9
Case	93 - 100	Scores 23, 29, 27 24, 39, 26 25, 40

indicating K.C.S.

Appendices to Chapters III, V and VI are recorded on magnetic computer tape, and are available on request from Dr. W.M. O'Brien, Department of Preventive Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22901, U.S.A.

APPENDIX CHAPTER IV

Orthoptic Patients (25)

Age	Sex
6	F
7	F
7	F
14	F
15	F
11	F
13	F
6	F
5	F
6	F
6	F
8	F
10	M
15	M
10	M
7	M
10	M
15	M
12	M
11	M
7	M
6	M
12	M
14	M
13	M

Orthopaedic and Accident Outpatients

(40 patients)

Age	Sex	Clinical State
16	F	Lacerations
23	F	Lacerations
30	F	Lacerations
29	F	Lacerations
27	F	Lacerations
24	F	Lacerations
17	F	Lacerations
22	F	Lacerations
18	F	Lacerations
18	F	Lacerations
18	F	Lacerations
25	F	Fractures
19	F	Fractures
30	F	Fractures
22	F	Fractures
24	F	Fractures
18	F	Fractures
16	F	Fractures
17	F	Fractures
19	F	Fractures
17	F	Fractures
20	F	Fractures
21	F	Fractures
29	M	Lacerations
17	M	Lacerations
16	M	Lacerations
24	M	Lacerations
26	M	Lacerations
27	M	Lacerations
28	M	Lacerations
24	M	Lacerations
16	M	Lacerations

Age	Sex	Clinical State
18	M	Lacerations
17	M	Lacerations
16	M	Fractures
16	M	Fractures
19	M	Fractures
22	M	Fractures
22	M	Fractures
25	M	Fractures

Miscellaneous Medical Clinics (120 patients)

Age	Sex	Clinical State	K.C.S.
35	M	Diabetes	
38	M	Diabetes	
74	M	Osteoarthritis	
61	M	Osteoarthritis	
58	M	Osteoarthritis	
52	M	Hernia	
59	M	Hernia	
76	M	Osteoarthritis	
78	M	Osteoarthritis	
64	M	Osteoarthritis	
74	M	Osteoarthritis	
71	M	Osteoarthritis	
36	M	Diabetes	
52	M	Diabetes	
51	M	Diabetes	
74	M	Osteoarthritis	
64	M	Osteoarthritis	
68	M	Osteoarthritis	
32	M	Migraine	
77	M	Osteoarthritis	
74	M	Osteoarthritis	
63	M	Osteoarthritis	
42	M	Migraine	
37	M	Migraine	
50	M	Migraine	
73	M	Arteriosclerosis	
41	M	Presbyopia	+
35	M	Diabetes	
59	M	Diabetes	
52	M	Diabetes	
62	M	Osteoarthritis	
64	M	Osteoarthritis	
66	M	Osteoarthritis	
67	M	Osteoarthritis	

Age	Sex	Clinical State	K.C.S.
40	M	Myopia	
72	M	Osteoarthritis	
73	M	Osteoarthritis	
61	M	Osteoarthritis	+
78	M	Arteriosclerosis	
44	M	Diabetes	
34	M	Diabetes	
33	M	Diabetes	
49	M	Diabetes	
50	M	Diabetes	
32	M	Diabetes	
78	M	Arteriosclerosis	
31	M	Hernia	
71	M	Hernia	
35	M	Hernia	
64	M	Hernia	
72	M	Hernia	
58	M	Hernia	
48	M	Hernia	
55	M	Hernia	
59	M	Hernia	
54	M	Hernia	
58	M	Hernia	
60	M	Hernia	+
63	F	Diabetes	
40	F	Diabetes	
63	F	Diabetes	
42	F	Diabetes	
48	F	Diabetes	
39	F	Myopia	
74	F	Arteriosclerosis	
64	F	Arteriosclerosis	
58	F	Arteriosclerosis	
59	F	Arteriosclerosis	
39	F	Presbyopia	
68	F	Arteriosclerosis	

Age	Sex	Clinical State	K.C.S.
52	F	Coronary	
44	F	Coronary	
38	F	Coronary	
59	F	Coronary	+
31	F	Hernia	
50	F	Coronary	
65	F	Coronary	
74	F	Arteriosclerosis	
72	F	Arteriosclerosis	
61	F	Diabetes	
49	F	Migraine	
71	F	Arteriosclerosis	
31	F	Migraine	
72	F	Arteriosclerosis	
63	F	Arteriosclerosis	
50	F	Cataract	
58	F	Cataract	
68	F	Cataract	
69	F	Cataract	
64	F	Cataract	
78	F	Arteriosclerosis	
48	F	Presbyopia	
68	F	Cataract	+
32	F	Diabetes	
58	F	Diabetes	
74	F	Arteriosclerosis	
60	F	Arteriosclerosis	
63	F	Arteriosclerosis	
44	F	Scoliosis	
52	F	Tuberculosis	
32	F	Tuberculosis	
72	F	Tuberculosis	
58	F	Tuberculosis	+
49	F	Tuberculosis	
63	F	Tuberculosis	
48	F	Tuberculosis	

Age	Sex	Clinical State	K.C.S.
78	F	Tuberculosis	+
61	F	Tuberculosis	
74	F	Tuberculosis	+
72	F	Tuberculosis	
59	F	Tuberculosis	
48	F	Tuberculosis	
43	F	Tuberculosis	
35	F	Tuberculosis	
39	F	Tuberculosis	
47	F	Tuberculosis	
48	F	Tuberculosis	
31	F	Tuberculosis	
74	F	Tuberculosis	
78	F	Tuberculosis	

GERIATRIC PATIENTS (145)

Clinical State - Cerebrovascular Disease

Age	Sex	K.C.S.	L.T.	A.N.F.	Total Protein	Alb.	Glob.	Alk. Phos.	Bilirubin	Glutamic Acid
80	M		+		7.4	3.2	4.2	6.8	0.46	27
80	M	+	+	+	8.0	4.0	4.0	8.4	0.48	28
81	M			+	7.2	3.8	3.4	8.6	1.00	29
80	M		+		7.0	3.6	3.4	8.2	0.40	28
82	M				7.0	3.6	3.4	8.8	1.20	32
80	M		+		6.8	3.4	3.4	9.0	1.00	34
84	M	+			8.0	3.6	4.4	8.4	1.2	30
80	M				7.4	3.8	3.6	8.4	0.46	34
86	M	+	+	+	7.6	4.2	3.4	8.0	0.48	30
95	M	+			7.0	3.6	3.4	6.8	0.48	22
81	M	+	+		7.8	4.2	3.6	6.8	0.44	20
82	M				7.0	3.4	3.6	7.0	0.40	26
84	M	+			7.0	3.4	3.6	7.0	0.60	26
83	M		+		7.0	3.6	3.4	7.8	0.60	26
88	M		+		7.2	3.8	3.4	7.8	0.50	26
89	M	+			7.2	4.0	3.2	8.2	0.62	28
92	M				7.6	4.6	3.0	8.4	0.50	28
80	M		+		7.8	4.8	3.0	8.0	0.48	28
81	M	+			8.0	4.6	3.4	8.0	0.44	26
82	M		+		8.0	4.6	3.4	9.4	0.46	26
83	M		+		8.0	4.2	3.8	9.0	0.44	24
84	M				7.4	3.4	4.0	9.0	0.48	20
81	M				7.4	3.6	3.8	5.8	1.0	20
82	M		+		7.6	3.6	4.0	6.0	1.2	20
90	M				7.6	4.2	3.4	6.2	0.54	20
91	M		+		7.4	3.0	4.4	6.4	0.50	20
80	M		+		7.0	3.8	3.2	6.8	0.48	28
81	M				6.8	3.8	3.0	7.2	0.46	22
82	M			+	6.8	3.8	3.0	7.2	0.44	24
83	M		+		6.8	3.6	3.2	7.4	0.42	26
84	M		+		7.0	3.8	3.2	7.8	0.40	26
87	M		+		7.2	4.0	3.2	7.8	0.48	28
86	M		+		6.8	3.4	3.4	8.0	0.48	28

Age	Sex	K.C.S.	L.T.	A.N.F.	Total Protein	Alb.	Glob.	Alk.Phos.	Bilirubin	Glutamic Acid
80	M	+			6.6	3.2	3.4	8.4	0.52	30
80	M				6.8	3.4	3.4	8.2	0.56	32
80	M		+		7.6	4.0	3.6	8.2	0.58	30
81	M		+		7.0	4.2	2.8	8.0	0.60	30
81	M		+		7.0	3.6	3.4	8.0	0.62	30
81	M				7.2	3.2	3.6	8.4	0.64	28
82	M			+	7.8	4.4	3.4	5.0	0.48	24
83	F				7.6	3.4	4.2	6.4	0.48	27
94	F				7.4	3.8	3.6	10.0	0.50	28
87	F	+	+	+	7.2	3.4	3.8	10.0	0.50	30
84	F			+	7.6	3.8	3.8	5.0	0.60	30
82	F	+			7.8	4.2	3.6	6.8	0.60	30
80	F		+		7.4	3.4	4.0	8.4	0.80	28
80	F		+		7.0	3.4	3.6	8.6	0.40	30
81	F				7.0	3.4	3.6	8.4	0.20	27
82	F	+	+		7.0	3.6	3.4	8.0	0.48	26
83	F				7.8	4.2	3.6	7.8	0.48	26
85	F		+	+	7.8	3.8	4.0	5.0	0.46	24
80	F		+	+	6.4	2.8	3.6	4.8	0.46	20
80	F		+	+	6.8	3.2	3.6	8.6	0.44	22
81	F				7.0	3.2	3.8	8.4	1.0	22
84	F				7.0	3.0	4.0	8.0	1.0	28
87	F				7.2	3.0	4.2	8.0	1.0	32
86	F				7.8	4.0	3.8	8.0	1.2	22
86	F	+			8.4	4.8	3.6	8.0	0.4	26
81	F		+	+	7.0	4.0	3.0	8.0	0.6	28
88	F		+	+	7.4	4.6	2.8	8.4	0.6	30
88	F		+	+	7.4	2.8	4.6	8.6	0.4	30
80	F	+			7.6	3.6	4.0	8.6	0.4	30
80	F	+			7.6	3.6	4.0	8.8	0.48	32
80	F	+			7.6	3.6	4.0	7.8	0.60	30
80	F				7.6	3.6	4.0	8.4	0.50	30
81	F				4.0	2.6	4.4	9.0	0.60	28
82	F	+	+	+	8.0	4.4	3.6	9.6	1.0	27
82	F		+		7.2	3.4	3.8	8.4	1.0	24

Age	Sex	K.C.S.	L.T.	A.N.F.	Total Protein	Alb.	Glob.	Alk. Phos.	Bilirubin	Glutamic Acid
82	F		+		7.0	3.6	3.4	8.4	0.48	24
82	F			+	7.0	3.6	3.4	8.6	0.46	24
82	F			+	7.2	2.4	4.8	5.0	0.44	24
83	F	+			8.0	4.6	3.4	10.0	0.30	26
84	F		+		7.4	3.8	3.6	8.0	0.38	26
82	F	+			7.4	3.8	3.6	9.0	0.46	26
83	F	+			7.6	3.2	4.4	4.6	0.44	24
86	F		+		7.4	3.6	3.8	4.6	0.44	24
86	F		+		7.0	3.2	3.8	9.0	0.42	22
82	F		+		7.4	3.2	4.2	5.6	0.40	22
82	F		+		7.6	3.4	4.2	5.8	0.40	30
86	F			+	7.6	3.4	4.2	6.0	0.38	28
85	F		+		7.4	3.4	4.0	8.6	0.44	23
84	F				7.6	3.8	3.8	8.4	0.46	32
83	F		+		7.8	3.4	4.4	8.2	0.44	30
86	F			+	8.2	3.4	4.8	8.0	0.48	25
88	F	+			8.4	5.0	3.4	8.6	0.48	28
87	F		+		7.0	3.4	3.6	8.2	0.30	26
81	F		+		7.4	4.2	3.2	7.6	0.28	26
82	F		+		7.6	4.8	2.8	7.8	0.26	26
88	F		+		7.4	3.2	4.2	8.0	0.24	26
87	F		+		7.4	4.0	3.4	8.6	0.20	26
84	F			+	8.0	5.6	3.4	8.8	0.18	26
84	F			+	8.2	5.6	3.4	8.6	0.44	26
84	F			+	7.6	4.2	3.4	8.0	0.46	25
85	F			+	7.6	4.2	3.4	8.6	0.44	26
80	F			+	7.4	4.0	3.4	8.4	0.42	26
81	F			+	7.8	4.4	3.4	8.4	0.40	26
80	F			+	7.4	4.0	3.4	8.0	0.46	25
81	F			+	7.0	3.4	3.6	7.8	0.44	24
80	F			+	7.0	3.4	3.6	7.8	0.42	24
80	F			+	7.0	3.4	3.8	6.2	0.40	32

Clinical State - Ischaemic Heart Disease

Age	Sex	K.C.S.	L.T.	A.N.F.	Total Protein	Alb.	Glob.	Alk. Phos.	Bilirubin	Glutamic Acid
81	M				7.0	3.6	3.4	5.8	0.38	23
83	M	+			7.0	4.0	3.0	7.8	0.36	20
82	M				7.2	4.2	3.0	5.8	0.38	24
81	F			+	7.0	4.0	3.0	5.0	0.36	28
82	F			+	7.8	4.4	3.4	9.0	0.40	28
84	F			+	7.6	4.4	3.2	8.4	0.44	20
85	F				7.4	3.0	4.4	8.0	0.46	20
87	F				7.6	3.0	4.6	7.8	0.48	20

Clinical State - Orthopaedic

86	M			+	7.8	3.4	4.4	5.0	0.44	20
80	M				7.0	3.2	3.8	4.8	0.48	32
80	M				7.8	4.2	3.6	6.2	0.44	20
81	M				8.2	4.0	4.2	10.0	0.48	28
80	F	+	+	+	8.0	3.2	4.8	6.4	0.50	24
81	F			+	7.8	3.0	4.8	10.2	0.54	26
82	F			+	7.6	3.4	4.2	10.2	0.60	24
80	F			+	7.6	3.4	4.2	4.8	1.0	20
80	F	+			7.6	2.6	5.0	5.6	1.0	32
81	F	+			7.6	2.6	5.0	5.8	0.48	30

Clinical State - Diabetes

84	F				7.4	3.0	4.4	8.0	0.50	32
84	F				7.6	3.4	4.2	8.4	0.50	30
81	F			+	7.6	3.6	4.0	9.2	1.0	30
87	M				7.6	3.4	4.2	8.0	0.44	27
80	M				7.4	3.0	4.4	8.2	0.46	28

Clinical State - Pernicious Anaemia

Age	Sex	K.C.S.	L.T.	A.N.F.	Total Protein	Alb.	Glob.	Alk. Phos.	Bilirubin	Glutamic Acid
88	F			+	7.4	4.0	3.4	8.2	0.46	34
93	F			+	7.2	3.6	3.6	8.0	0.46	32
94	F			+	7.4	4.0	3.4	8.2	0.38	30
89	F			+	7.0	3.2	3.8	7.8	0.30	20
87	F				7.0	4.0	3.0	7.8	0.28	28

Clinical State - Nutritional Anaemia

80	M				7.0	3.6	3.4	8.0	0.48	20
87	M				7.0	3.4	3.6	7.6	0.44	24
84	M				7.6	3.8	3.8	8.0	0.46	20
81	M				7.4	3.2	4.2	7.6	0.48	28
83	M				7.0	3.0	4.0	7.4	0.50	27

Clinical State - Hypertension

82	F	+			7.6	3.6	4.0	8.0	0.60	27
86	F				7.6	2.6	5.0	8.2	0.60	30
88	F				7.6	2.6	5.0	8.6	0.60	32
89	F				7.8	3.4	4.4	8.6	0.64	32

Clinical State - Parkinson's Disease

90	M				7.8	4.4	3.4	8.0	0.70	20
91	M				8.0	4.2	2.8	8.0	1.2	32

Age	Sex	K.C.S.	L.T.	A.N.F.	Total Protein	Alb.	Glob.	Alk. Phos.	Bilirubin	Glutamic Acid
Clinical State - Rodent Ulcer										
93	M				7.0	3.6	3.4	7.8	1.0	30
Clinical State - Gangrene										
94	M				7.4	4.4	3.2	8.0	1.0	30
Clinical State - Paget's Disease										
98	F			+	7.4	4.0	3.4	7.8	0.80	28
Clinical State - Obesity										
91	M				7.2	3.6	3.6	7.8	0.46	26
84	F			+	7.4	3.6	3.8	7.8	0.46	26
Clinical State - Hypothyroidism										
80	F				7.4	3.6	3.8	8.0	0.44	24

APPENDIX CHAPTER VII

Hashimoto's Disease (all female)

Age	Clinical Status	Thyroid Autoantibody Tests: Prec.Test	T.R.C.Test	Fluor.'C.F.'Test	Comments	S.T.	K.C.S.
57	H	P	Neg.	++		11	
75	H	P	Neg.	++			
47		P	Neg.	+			
70		Neg.	Neg.	++	B	12	
51		P	Neg.	++			
74		P	Neg.	++			
50		P	Neg.	+		11	
64		Neg.	4,000	+	B	12	
54		P	Neg.	Neg.			
62		P	Neg.	++			
42		P	4,000	+		3	+
37		P	Neg.	Neg.			
43		P	256	Neg.			
82		Neg.	Neg.	Neg.	B		
48		P	4,000	Neg.			
59		P	16	+			
50		P	Neg.	++			
58		P	4,000	+			
86		P	4,000	+		8	+
43		P	Neg.	Neg.			
61		P	Neg.	Neg.			
42		Neg.	Neg.	Neg.	B		
63		Neg .	1,000	++	B		
50		P	Neg.	++			
60		P	Neg.	Neg.			
72		P	256	+		12	
61		P					
51		P	4,000	++			
54		P	1,000	++			
54		P	64	++			
68		P	64	Neg.		10	
57		P	256	+			
66		P	Neg.	Neg.		9	

Age	Clinical Status	Thyroid Autoantibody Tests					
		Prec.Test	T.R.C.Test	Fluor.'C.F.'Test	Comments	S.T.	K.C.S.
45		P	Neg.	+		4	+
63		P	1,000	Neg.	R.A.	11	
69		P	Neg.	Neg.	R.A,		
66		P	64	++	R.A.		
61		P	16	++	R.A.		
63		P	16	++			
44		P	Neg.	++		2	
60		P	Neg.	++			
37		P	1,000	++			
55		P		++			
67		P		++		5	
66		P		++			
63		P		++			
63		P		++			
74		P				11	
71		P				5	
54		P		+			
41		P		++			
56		P		++			
50		P		++		7	
38		P		++			
50		P		++		8	
47		P		Neg.			
48		P		+			
54		P		+			
55		P		++			
41		P		++		6	
39		P		++			
40		P		++			
40		P		++			
40		P		++			
40		P		++			
40		P				8	
40		P		++			
51		P				4	
49		P				7	

Age	Clinical Status	Thyroid Autoantibody Tests: Prec.Test	T.R.C.Test	Fluor.'C.F.'Test	Comments	S.T.	K.C.S.
50		P					
51		P					
49		P					
50		P					
51		P					
49	H	P					
50		P				9	
60		P				3	
61		P					
59		P					
60		P					
61		P					
59		P					
59		P					

Hypothyroidism (female patients)

Age	Clinical Status	Thyroid Autoantibody Tests: Prec.Test	T.R.C.Test	Fluor.'C.F.'Test	Comments	S.T.	K.C.S.
59	H						
71				Neg.			
63		P		++			
48							
74	H			Neg.			
59	H			++			
67				Neg.			
50							
59	H						
58	H	P		Neg.			
55	H						
64				++			
66				++			
69							
63				+			
53				+			
81	H						
63	H						
56				Neg.			
60							
68				Neg.			
42				+++			
74		P					
51		P		Neg.			
62				+			
63							
62							
53				++			
46				Neg.			
56							
66				Neg.			
44				Neg.			
33							
50			Neg.	Neg.			

Age	Clinical Status	Thyroid Autoantibody Tests: Prec.Test	T.R.C.Test	Fluor.'C.F.'Test	Comments	S.T.	K.C.S.
46						4	
48				Neg.		8	
54				++			
53				++		3	+
67				+		8	
62				+		3	+
64				+		11	
67						5	
68				Neg.		6	
63	H			Neg.			
67				+		4	
40				Neg.		8	
65				Neg.		5	
55				Neg.			
53				++		2	+
49				+		8	
51				+		12	
73	H						
66		P		+		0	+
53				Neg.	R.A.		
62		P		++	R.A.	12	
58		P		Neg.	R.A.		
61				+	R.A.		
73						6	
50				+		11	
50				+		12	
56							
51						0	+
53							
50						4	
52							
51						5	
52							
51						6	
53							

Simple Goitre (all female)

Age	Thyroid Autoantibody Tests				
	Prec.Test	T.R.C.Test	Fluor.'C.F.'Test	S.T.	K.C.S.
44	Neg.	Neg.	Neg.		
28	Neg.				
31	Neg.		Neg.		
42					
21					
35					
56		1:16	+		
20					
53					
37					
47					
32					
37					
42					
25					
25					
43					
16					
19					
33		1:16		11	
31					
21				10	
39					
25				13	
32					
47				10	
32					
33					
67					
37					
46					
50					
39				14	

Thyroid Autoantibody Tests

Age	Prec.Test	T.R.C.Test	Fluor.'C.F.'Test	S.T.	K.C.S.
18					
53					
36					
47				4	+
40					
15					
63					
62					
21					
19					
57				4	+
42					
61					

PERNICIOUS ANAEMIA MALE PATIENTS (37)

	1	2	3	4	5	6	7	8	9	10
Age	65	80	62	55	44	63	70	55	66	60
Comments Family History				+		+		0		
S.T.		7	4		13			14		
K.C.S.										
P.T.G.	+		+		+			+		
Hb.	11	10	12	13	11	9	8	10	13	11
P.C.A.	+		+	+	+		+	+	+	+
T.A.			+			+	+			
T.R.C.		+		+			+		+	+

	11	12	13	14	15	16	17	18	19	20
Age	71	62	20	55	75	60	82	60	80	70
Comments Family History	0			+		+				
S.T.	12		11		10		8		8	
K.C.S.										
P.T.G.			+		+	+				
Hb.	11	11	11	12	11	11	11	10	11	13
P.C.A.	+	+	+		+	+				
T.A.										
T.R.C.										

	21	22	23	24	25	26	27	28	29	30
Age	60	65	70	72	70	63	45	80	60	75
Comments Family History		0						+		0
S.T.				7		1		11		
K.C.S.						+				
P.T.G.	+	+				+	+			
Hb.	13	11	11	10	11	11	12	13	13	12
P.C.A.										
T.A.										
T.R.C.										

	31	32	33	34	35	36	37
Age	68	70	78	55	74	70	66
Comments Family History			+				
S.T.	4				12		
K.C.S.							
P.T.G.				+			+
Hb.	11	11	11	10	11	12	12
P.C.A.							
T.A.							
T.R.C.							

Pernicious Anaemia Female Patients (40)

	1	2	3	4	5	6	7	8	9	10
Age	65	56	68	64	72	68	65	70	65	58
Comments Family History						O			O +	
S.T.	8	7	10	5	11	7	9		4	
K.C.S.				+					+	
P.T.G.	+			+		+				+
Hb.	12	11	13	11	10	9	11	8	13	11
P.C.A.	+	+	+	+		+	+	+		+
T.A.	+	+		+		+		+		+
T.R.C.				+	+		+			+

	11	12	13	14	15	16	17	18	19	20
Age	65	51	65	63	26	44	68	59	52	70
Comments Family History	+			+			O			+
S.T.	8	5		13		14		4		8
K.C.S.		+								
P.T.G.				+						+
Hb.	11	11	11	11	12	12	11	11	11	11
P.C.A.		+		+	+	+	+		+	+
T.A.	+			+	+		+	+	+	
T.R.C.			+			+		+		

	21	22	23	24	25	26	27	28	29	30
Age	66	67	70	70	63	46	48	70	59	60
Comments Family History							+	+		
S.T.			9	8		7	4	6		0
K.C.S.										+
P.T.G.							+			
Hb.	11	11	11	11	11	12	13	14	11	10
P.C.A.	+	+	+	+	+	+	+			
T.A.		+	+	+		+				
T.R.C.										

	31	32	33	34	35	36	37	38	39	40
Age	68	69	48	50	71	50	52	48	60	67
Comments Family History		0		+		+				
S.T.			1		12			2		
K.C.S.			+							
P.T.G.	+		+			+		+	+	
Hb.	13	11	11	11	11	11	11	11	11	11
P.C.S.										
T.A.										
T.R.C.										

Idiopathic Addison's Disease

20 patients

Age	30	45	40	41	44
	42	34	40	20	37
	39	44	38	38	33
	39	41	40	42	36

Hospital Control Patients - see Appendix to Chapter IV.

APPENDIX CHAPTER VIII

BACTERIOLOGICAL STUDIES

60 Arthritic Patients - Clinically Healthy Eyes

(Patients 1-5 male; Remainder female)

Patient	Sacs	Lids	Nose
1	+S	+S	+
2	+	+	+
3		+	+S
4	+	+	
5		+	+
6	+	+	+
7	+	+	+
8	+	+	+
9	+	+	
10	+		+
11	+S	+S	
12	+	+	+
13			+
14		+S	+S
15	+	+	
16	+	+	
17		+S	
18	+S	+	
19	+	+	
20	+	+	+
21	+	+	+
22	+	+	+
23	+	+	+
24	+	+	+
25	+S	+	+
26			+S
27		+S	
28		+S	
29		+S	
30			+S

Patient	Sacs	Lids	Nose
31	+	+	
32	+	+	
33	+	+	+
34		+	+S
35		+	+S
36			+S
37	+	+	+
38	+S	+S	+S
39	+S	+	
40	+	+	+
41	+	+	+
42	+	+	+
43	+	+	
44			+S
45		+	
46	+	+	
47	+	+	
48	+	+	
49	+	+	
50	+	+	+
51	+	+	+
52			+
53			+S
54			+S
55			+S
56			+S
57		+S	+
58			+
59			+
60			+S

65 Untreated Keratoconjunctivitis Sicca Patients

Patient	Sacs	Lids	Nose	C.I.
1	+S	+S	+S	+
2	+S	+S	+S	+
3	+S	+S	+S	+
4	+S	+	+	
5	+	+S	+	
6	+	+S	+	
7	+		+	
8	+S	+S	+S	+
9		+S	+	
10	+S	+	+	
11		+S	+	
12	+	+S	+	
13	+		+	
14	+S	+S	+	+
15		+S	+	+
16	+S	+S	+	+
17	+	+S	+	
18	+	+S		
19	+S	+S	+S	+
20	+S	+		
21	+	+S		
22	+		+	
23	+S	+S	+S	+
24	+S	+S	+	+
25	+	+		
26	+	+S	+S	+
27	+	+S	+S	+
28	+	+S	+S	+
29	+	+S	+S	+
30	+	+S		+
31	+S	+S		+
32	+S	+	+	
33	+	+S	+	+

Patient	Sacs	Lids	Nose	C.I.
34	+	+S	+	+
35	+	+S	+	+
36	+	+S		
37	+	+S		
38	+	+S		
39	+S	+		+
40	+S	+		+
41	+	+S	+	+
42	+	+S	+	+
43	+	+S	+	+
44	+	+S		
45	+S	+		
46	+	+S		+
47	+S	+		
48	+S	+	+S	+
49	+S	+	+S	+
50	+	+S		+
51	+	+S		+
52	+S	+	+S	+
53	+S	+		
54		+	+S	
55	+	+	+S	
56	+S	+		
57	+S	+		+
58		+	+S	
59	+S	+	+	+
60	+S	+	+	+
61	+S	+	+	+
62	+S	+	+	+
63	+S	+		+
64		+S		+
65	+	+S		

65 Keratoconjunctivitis Sicca Patients After One Month of Tear Substitute Therapy

Patient	Sacs	Lids	Nose
1	+S	+S	+S
2	+S		+S
3	+S		+S
4		+	+
5		+S	+
6		+S	+
7		+	
8	+S		+S
9		+	
10	+S	+	+
11		+S	+
12		+S	+
13		+	
14	+S	+	+
15		+S	+
16	+S	+	+
17		+S	+
18		+	+
19		+S	+S
20		+	
21		+	
22			
23	+S		+S
24	+S		
25			+
26		+S	
27			+S
28			+S
29		+S	+S
30			+
31	+S		
32			+
33			+

Patient	Sacs	Lids	Nose
34		+S	
35			+
36		+S	
37		+	+S
38		+	+S
39		+	
40			+
41		+S	+
42		+S	+
43		+S	+
44		+	+
45		+	
46		+	
47			+
48		+	+S
49		+	+S
50			+
51			
52	+S		
53		+	
54		+	+S
55		+	
56		+	
57		+	
58		+	+
59		+	+
60		+	+
61			+
62	+S		
63			+S
64			
65			

S = staph. aureus

Clinically Healthy Group

(Patients' numbers adjusted for ease of recording)

Patient No.	Age	Sex	Culture	
1-39		M	Negative	Age Group - 0-9 yrs.
40-56		F	Negative	
57	8	F	Isaria	
58-160		M	Negative	Age Group - 10-19 yrs.
161	12	M	Aspergillus	
162	18	M	Candida	
163-262		F	Negative	
263	19	F	Candida	
264	17	F	Papulospora	
265	14	F	Saccharomyces	
266	16	F	Rhizopus	
267-287		M	Negative	Age Group - 20-29 yrs.
288	26	M	Penicillium	
289-308		F	Negative	
309	26	F	Rhodotorula	
310	28	F	Rhodotorula	
311	20	F	Scopulariopsis	
312-330		M	Negative	Age Group - 30-39 yrs.
331-352		F	Negative	
353	37	F	Penicillium	
354-378		M	Negative	Age Group - 40-49 yrs.
379	46	M	Penicillium	
380	41	M	Penicillium	
381-400		F	Negative	
401	40	F	Penicillium	
402	45	F	Glioclachium	

Patient No.	Age	Sex	Culture	
403-428		M	Negative	Age Group - 50-59 yrs.
429	56	M	Penicillium	
430	57	M	Aspergillus	
431-452		F	Negative	
453	55	F	Aspergillus	
454	54	F	Aspergillus	
455	58	F	Scopulariosis	
456	59	F	Nigrospora	
457-484		M	Negative	Age Group - 60-69 yrs.
485	63	M	Penicillium	
486	61	M	Rhodotorula	
487	60	M	Candida	
488	64	M	Isaria	
489	66	M	Geotrichum	
490	67	M	Geotrichum	
491-510		F	Negative	
511	65	F	Geotrichum	
512	66	F	Hormodendrin	
513-533		M	Negative	Age Group - 70+ yrs.
534-551		F	Negative	
552	75	F	Scopulariopsis	
553	78	F	Isaria	

Healthy Eyes Before and After One Week Steroid and Steroid/Antibiotic

Betamethasone disodium phosphate

Patient	1st Culture	2nd Culture
1-21	Negative	Negative
22	Aspergillus	Negative
23-46	Negative	Negative
47	Rhodotorula	Negative
48-61	Negative	Negative
62	Negative	Aspergillus
63	Negative	Penicillium
64-81	Negative	Negative
82	Negative	Saccharomyces
83-86	Negative	Negative

Betamethasone disodium phosphate/Neomycin sulphate

Patient	1st Culture	2nd Culture
1	Candida Alb.	Candida Alb.
2-10	Negative	Negative
11	Negative	Candida Alb.
12	Negative	Penicillium
13-26	Negative	Negative
27	Negative	Candida Alb.
28-38	Negative	Negative
39	Negative	Penicillium
40	Negative	Aspergillus
41	Negative	Penicillium
42	Negative	Streptomyces
43-48	Negative	Negative
49	Negative	Aspergillus
50	Negative	Trichoderma
51	Negative	Negative
52	Negative	Aspergillus

Patients with External Ocular Disease Receiving Topical Steroid or Steroid/Antibiotic

Betamethasone disodium phosphate

Patient	1st Culture	2nd Culture
1-43	Negative	-
44	Penicillium	Negative
45-46	Negative	-

Betamethasone disodium phosphate/Neomycin sulphate

Patient	1st Culture	2nd Culture
1-44	Negative	-
45	Penicillium	Negative
46	Aspergillus	Negative
47-50	Negative	-
51	Scopulariopsis	Negative
52-54	Negative	-
55	Rhodotorula	Negative

Sjøgren Syndrome Patients

Receiving Systemic Steroids

Patient	1st Culture	1st Repeat of +ve Cultures	2nd Repeat of +ve Cultures
1	Penicillium	Negative	Negative
2	Rhodotorula	Negative	Aspergillus
3	Rhodotorula	Negative	Rhodotorula and Candida Alb.
4	Candida Alb.	Negative	Not repeated.
5-14	Negative	-	-

Not Receiving Systemic Steroids

Patient	1st Culture	1st Repeat of +ve Cultures	2nd Repeat of +ve Cultures
1	Stemphylium	Negative	Candida Alb.
2	Rhodotorula	Negative	Penicillium
3	Candida Alb.	Negative	Candida Alb.
4-10	Negative	-	-
11	Tropicalis	Negative	Parapsilosis
12	Candida Alb.	Negative	Candida Alb.
13-20	Negative	-	-
21	Hormiscum	Negative	Not repeated
22-23	Negative	-	-

APPENDIX CHAPTER IX

Patients 1 - 8 are the same as patients 33 - 40 Appendix II

Patients 11 - 42 are the same as patients 1 - 32 Appendix II

3 Years of BJ6 Therapy - 65 Patients Completed Course

Age	Sex	Symptoms	Signs	Both	Selection N-L.	Acet.	Cyst.
54	M	+	+	+			
54	M	+	+	+			
56	M	+	+	+			
57	M	+	+	+			
61	M	+	+	+			
63	M	+	+	+			
65	M	+	+	+			
67	M	+	+	+			
54	M	+	+	+			
68	M	+	+	+			
62	F	0	0	0	X		X
61	F	+	+	+			
48	F	+	-				X
56	F	+	+	+			
52	F	+	+	+			
59	F	+	+	+			
60	F	0	0	0	X		
62	F	+	-				X
49	F	+	0				
53	F	-	0				
54	F	+	+	+			
59	F	-	0				X
61	F	0	0	0	X		X
62	F				X		X
51	F	0	0	0	X		
56	F	+	+	+			
50	F				X		
48	F				X		
64	F	+	+	+			
66	F	+	+	+			
49	F	+	+	+			
52	F						X
57	F	+	+	+			
54	F	+	+	+			

Age	Sex	Symptoms	Signs	Both	Selection N-L.	Acet.	Cyst.
53	F	+	+	+			
59	F	+	+	+			
48	F	+	+	+			
32	F				X		
49	F				X		
58	F				X		
41	F				X		
54	F	+	+	+			
68	F				X		
64	F				X		
61	F	+	-				X
54	F	+	-				X
38	F				X		
55	F				X		
54	F						X
66	F	0	0	0	X		
67	F	0	0	0	X		
70	F				X		
55	F				X		
59	F				X		
54	F	+	-				X
56	F	+	-				X
56	F				X		
56	F				X		
48	F				X		
39	F				X		
62	F	+	+	+			
50	F	+	+	+			
64	F	+	+	+			
49	F	+	+	+			
66	F	0	-				X
57	F				X		X
54	F	+	+	+			

Age	Sex	Symptoms	Signs	Both	Selection N-L.	Acet.	Cyst.
56	F				X		
54	F	+	+	+			
63	F	0	-				
65	F				X		X
68	F	+	+	+			
54	F				X		
68	F	+	+	+			
62	F	+	+	+			
61	F				X		X
56	F				X		
61	F	+	+	+			
62	F				X		X
59	F				X		
54	F				X		X
52	F						X
57	F	+	+	+			
48	F						X
58	F	+	+	+			
60	F	+	+	+			
63	F				X		
62	F						X
45	F	+	+	+			
66	F	+	+	+			
44	F	-	+				X
71	F	-	-	-			X
74	F	-	-	-			X
68	F	+	+	+			
64	F	+	+	+			
54	F	+	+	+			
56	F	+	+	+			
59	F	+	0				

Naso-lacrimal Duct Obliteration - 32 Patients All Female

Age	Symptoms	Signs	Both	Selection Acet. Cyst.
62	+	+	+	
60	+	+	+	
61	-	-	-	X
62	+	+	+	
51	+	+	+	
50	+	-		X
48	+	-		X
32	+	+	+	
49	+	+	+	
58	+	+	+	
41	-	-	-	X
68	+	+	+	
64	+	+	+	
38	+	+	+	
55	+	-		
66	+	+	+	
67	+	+	+	
70	+	+	+	
55	+	+	+	
59	+	-		X
56	+	+	+	
56	+	+	+	
48	+	+	+	
39	-	-	-	X
57	+	+	+	
56	-	+		
65	+	-		
54	+	-		
61	-	-		
56	0	0	0	X
62	0	0	0	X
59	+	+	+	

6 Months of Acetyl-Cysteine Therapy -

20 Patients All Female

Age	Symptoms	Signs	Both
61	0	0	0
50	+	+	+
48	-	+	
41	+	+	+
59	+	+	+
39	-	+	
56	+	+	+
62	+	-	
48	+	+	+
59	-	-	-
61	+	+	+
62	-	-	-
61	-	-	-
54	-	-	-
54	-	-	-
56	-	-	-
66	-	-	-
57	-	-	-
62	-	-	-
44	-	-	-

EFFECT OF TEMPERATURE AND HUMIDITY IN THE SCHIRMER TEAR TEST

BY

J. WILLIAMSON AND M. ALLISON

Reprinted from British Journal of Ophthalmology
Volume 51, *No.* 9, *pages* 596–598, *September*, 1967

LONDON
BRITISH MEDICAL ASSOCIATION
TAVISTOCK SQUARE, W.C.1

Brit. J. Ophthal. (1967) **51**, 596

EFFECT OF TEMPERATURE AND HUMIDITY IN THE SCHIRMER TEAR TEST*†

BY

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THE Schirmer I tear test developed and standardized by Halberg and Berens, New York City (Contactisol Inc. Lindenhurst, N.Y.) is a useful screening procedure for keratoconjunctivitis sicca in large numbers of patients suffering from various connective tissue disorders.

When all the patients attending a centre for rheumatic diseases were subjected to this test it was noted that a surprisingly high percentage of in-patients had positive Schirmer I readings (less than 15 mm.). At the time of writing, 338 in-patients (age range 18 to 72 years, mean 56) including 250 females and 88 males, had been examined. In 65 patients (20 per cent.) whose ages ranged from 25 to 70 years (mean 50·5), 123 eyes had shown apparently reduced tear flow, 78 producing less than 5 mm. of wetting. When, however, the test was repeated within the week at an ophthalmic out-patients department, all the results were negative.

The 65 patients were tested the following week in the rheumatic hospital and tear flow was again apparently reduced in 110 eyes.

Investigations

A thermographic recording and simultaneous measurements of the humidity using a whirling string hygrometer (wet and dry bulb), were made in both hospitals over a period of 20 days.

Fig. 1 (opposite) shows part of the tracing from the rheumatic hospital, where the mean temperature was 72·3°F. (22·3°C.), and the humidity 40·5.

Fig. 2 (opposite) shows part of the tracing from the eye hospital, where the mean temperature over the same period was 57·4°F. (14·1°C.), and the humidity 48.

During the recording time, twelve of the patients (age range 40 to 58 years; mean 49) with apparently reduced tear flow were moved between the two hospitals on three occasions. Six recordings of the Schirmer I tear test were made available in each eye, three from the rheumatic centre and three from the ophthalmic hospital. The Table (opposite) shows the results.

All had rose bengal 1 per cent. instilled into the conjunctival sac and were examined by a modern Zeiss slit-lamp. None of the patients had keratoconjunctivitis sicca.

Clearly the Schirmer tear test was consistent in each hospital, but it was always consistently lower in the higher temperature and lower humidity of the rheumatic wards.

An interesting feature of this experiment was that the tear flow was not noticeably reduced until after 12 hours' in-patient stay in the rheumatic wards.

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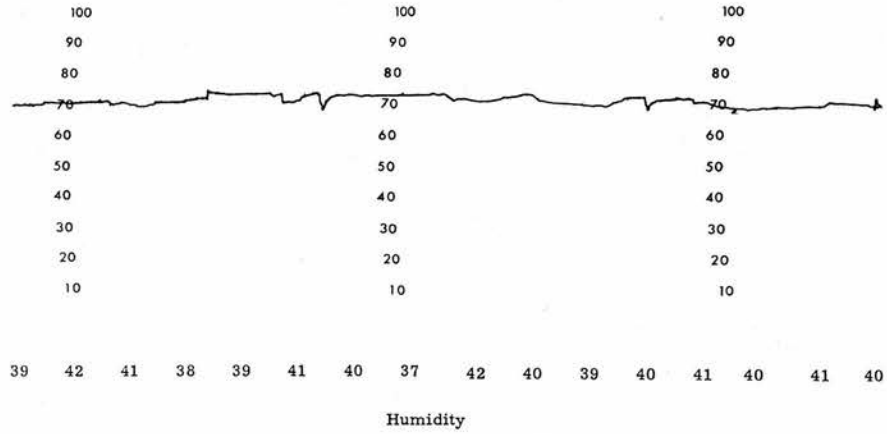

FIG. 1.—Thermograph recording in a rheumatic hospital.

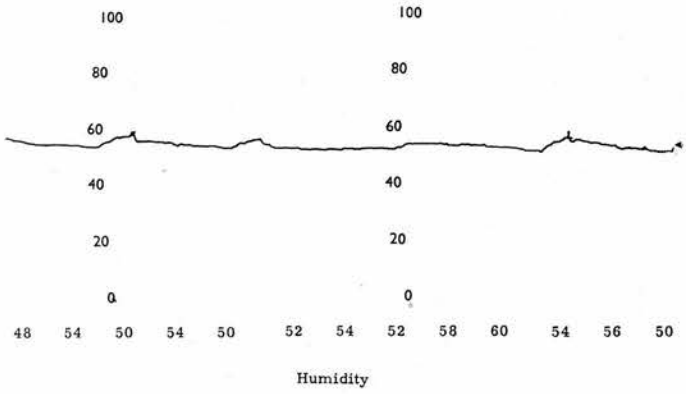

FIG. 2.—Thermograph recording in an ophthalmic out-patients department.

TABLE

Case No.	Sex	Three Recordings (average of two eyes)	
		At Rheumatic Hospital	At Ophthalmic Hospital
1	F	11, 12, 11	15, 18, 18
2	F	8, 7, 7	20, 20, 19
3	M	4, 8, 7	16, 18, 17
4	F	3, 7, 5	20, 18, 20
5	F	8, 12, 11	20, 20, 20
6	M	12, 11, 12	22, C.W., C.W.
7	M	14, 15, 10	18, 18, 20
8	M	15, 11, 14	19, 20, 20
9	M	14, 12, 13	22, C.W., C.W.
10	F	12, 9, 8	20, 18, 16
11	M	7, 6, 4	20, 16, 20
12	F	3, 2, 0	20, C.W., 20

(C.W. = completely wet)

The twelve patients were then followed up during the next 20 days, the Schirmer I test being repeated every second night. During this time, there was no tendency for the patients to compensate by increased production of tears, *i.e.* the results remained consistently low. Within 3 days of hospitalization, ten of the patients complained that their eyes felt hot and dry towards early afternoon, and within the week so did the rest.

Discussion

65 out of 338 patients (20 per cent.) admitted to a rheumatic centre showed apparently reduced tear flow (less than 15 mm.) after 12 hours' hospitalization, as judged by the Schirmer I tear test. All were measured within the week at an ophthalmic out-patients department and were found to have normal tear flow. The findings appear to be related to the differences in temperature and humidity in the two hospitals. Twelve of the patients were repeatedly measured in both departments and the results were consistent. Over a period of 20 days, no tendency to compensate for the high temperature and low humidity in the rheumatic wards was observed. Symptoms of dry eyes developed in 3 days in ten of the patients and in all within the week.

A number of interpretations may be put on this observation. The apparently reduced tear flow may have been the result of increased evaporation in the warm, dry rheumatic wards. If this were correct, however, then the apparent diminution in tear flow would not have taken 12 hours to develop from the time of admission. Further, it may be asked why it should have affected only 20 per cent. of the patients and not all of them and why this effect remained consistent for this selected group of in-patients.

If the tear flow was actually increased for emotional or other reasons during the first 12 hours of admission, this might account for the apparently normal Schirmer I tear tests.

On the other hand, there may be a true lack of response to dry heat in some individuals, *i.e.* these findings may be physiological. The only way to prove this would be to admit a group of patients, suffering neither from connective tissue disorders nor from any disease believed to have an immunological basis, to the rheumatic centre for several days. Clearly this is impractical.

Finally, the lack of response to temperature and/or humidity may occur only or more readily in patients who are likely to develop keratoconjunctivitis sicca at a later date. It will be extremely interesting, therefore, to follow these patients over the next decade.

Summary

A temperature of around 72°F. (22°C.) and/or humidity of around 40·5 results in a reduced Schirmer I tear test after 12 hours of in-patient stay in about 20 per cent. of patients suffering from connective tissue disorders. No tendency to compensate for this reduction was noted in twelve patients over 20 days despite the fact that they developed symptoms of dry eyes. The possible significance of this observation is discussed.


Printed in England by Staples Printers Limited at their Kettering, Northants, establishment

SJÖGREN'S SYNDROME AND THYROID DISEASE

BY

J. WILLIAMSON, J. STANLEY CANT, D. K. MASON,
W. R. GREIG, AND J. A. BOYLE

Reprinted from British Journal of Ophthalmology
Volume 51, *No.* 11, *pages* 721–726, *November*, 1967

LONDON
BRITISH MEDICAL ASSOCIATION
TAVISTOCK SQUARE, W.C.1

Brit. J. Ophthal. (1967) **51**, 721

COMMUNICATIONS

SJÖGREN'S SYNDROME AND THYROID DISEASE*†

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SJÖGREN'S syndrome is a chronic benign systemic disease of unknown aetiology which involves principally the lacrimal and salivary glands and may occasionally also affect the naso-pharyngeal, buccal, oesophageal, and tracheo-broncheal glands and the sweat glands (Sjögren, 1943, 1951; Stoltze, Hanlon, Pease, and Henderson, 1960; Bloch, Buchanan, Wohl, and Bunim, 1965). The majority of patients studied have underlying connective tissue disease such as rheumatoid arthritis, systemic lupus erythematosus, progressive systemic sclerosis, polymyositis, or polyarteritis nodosa (Bloch and Bunim, 1963; Bloch and others, 1965), each of which is characterized by circulating autoantibodies to cells or cell constituents.

Anderson, Gray, Beck, and Kinnear (1961); Bunim (1961); Bloch and Bunim (1963); Anderson, Beck, Bloch, Buchanan, and Bunim (1965), have also shown that the prevalence of thyroglobulin autoantibodies is increased in patients with Sjögren's syndrome, but the corresponding prevalence of Sjögren's syndrome in patients with autoimmune thyroid disease has not been evaluated. The histological features of autoimmune thyroiditis, Hashimoto's thyroiditis, and spontaneous myxoedema and those of the lacrimal and salivary glands in Sjögren's syndrome are, however, very similar, as first noted by Hashimoto (1912). For this reason it appeared to be important to determine whether the two conditions occur together with significant frequency. Since thyroid autoantibodies are also found in patients with thyrotoxicosis (Roitt and Doniach, 1958; Buchanan, Alexander, Crooks, Koutras, Wayne, Anderson, and Goudie, 1961; Buchanan, Koutras, Crooks, Alexander, Brass, Anderson, Goudie, and Gray, 1962; Anderson, Gray, Middleton, and Young, 1964), the incidence of Sjögren's syndrome in this disorder was also determined. In each group of patients the incidence of Sjögren's syndrome was compared with that in a group with simple goitre and that in another control group of hospital patients.

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Lacrimal gland function is difficult to assess accurately even where the gland is healthy, and routine biopsy is not a practical procedure; the most reliable clinical indication of lacrimal gland involvement is the presence of kerato-conjunctivitis sicca which is readily diagnosed and this is the index we have used in the investigation. Examination of the salivary glands was also made in a large number of patients by means of sialography and the incidence of abnormalities was determined.

Material and Methods

Patients

339 female patients comprising five groups were examined for kerato-conjunctivitis sicca. The mean age and age range are shown in Table I.

TABLE I

KERATO-CONJUNCTIVITIS SICCA IN THYROID DISEASE

Clinical Groups	Number of Patients	Age (yrs)		Schirmer's Test (mm. at 5 minutes)						Kerato-conjunctivitis Sicca	
		Mean	Range	5		5–9		10–14		No.	Per cent.
				No.	Per cent.	No.	Per cent.	No.	Per cent.		
Hashimoto's Thyroiditis	83	55·0	37–75	5	6·0	11	13·3	7	8·4	3	3·6
Primary Hypothyroidism	69	67·0	33–74	6	8·7	12	17·3	5	7·2	5	7·2
Thyrotoxicosis	68	43·2	16–66	7	10·3	1	1·4	—	—	6	8·6
Simple Goitre	46	39·5	15–67	2	4·3	—	—	5	11·0	2	4·3
Hospital Controls	72	51·9	31–74	5	6·9	10	13·9	6	8·3	5	6·9

Group 1. Hashimoto's Thyroiditis (*83*).—The diagnosis was based on five patients on histological examination of the gland using the criteria of Joll (1939) and in the remaining 78 on the presence of a positive precipitin test for antithyroglobulin autoantibodies in a euthyroid or hypothyroid patient (Buchanan and others, 1961). Three of these patients were hypothyroid when studied, but the remaining eighty were receiving 0·2 mg. sodium thyroxine and were euthyroid when studied. Four had "definite" rheumatoid arthritis by the American Rheumatism Association criteria (Ropes, Bennett, Cobb, Jacox, and Jessar, 1958).

Group 2. Spontaneous Primary Hypothyroidism (*69*) (hypothyroidism without a goitre).—The diagnosis was based on the clinical and laboratory criteria described by Wayne (1960) and Wayne, Koutras, and Alexander (1964). Ten patients were hypothyroid when examined and the remaining 59 were receiving 0·2–0·3 mg. sodium thyroxine and were euthyroid. Four had "definite" rheumatoid arthritis by the American Rheumatism Association criteria (Ropes and others, 1958).

Group 3. Thyrotoxicosis (*68*).—21 patients had become hypothyroid following radioactive iodine (^{131}I) therapy; twenty of them were euthyroid at the time of examination and were receiving 0·2–0·3 mg. sodium thyroxine per day and the other was hypothyroid. The remaining 47 were euthyroid following antithyroid drug therapy, subtotal thyroidectomy, or ^{131}I therapy.

Group 4. Simple Goitre (*46*).—All these were euthyroid, and none had systemic disease.

Group 5. Hospital Controls (*72*).—These were all women attending as outpatients at the clinics associated with the Western and Royal Infirmaries, Glasgow. They had a variety of general medical conditions, none of which, however, had any known association with thyroid disease or autoimmune disorders.

Methods

Autoantibodies to Thyroglobulin were tested by a precipitin test using the Ouchterlony-Elekplate technique (Anderson, Buchanan, Goudie, and Gray, 1962) and by the tanned red cell haemagglutination test using thyroglobulin-coated formalized tanned sheep red cells (Burroughs Wellcome), (Fulthorpe, Roitt, Doniach, and Couchman, 1961). The lowest serum dilution tested in the tanned red cell haemagglutination test was 1 in 16. Autoantibody to thyroid "microsomes" was measured by an immunofluorescence technique on unfixed frozen sections of thyroid slices (Holborow, Brown, Roitt, and Doniach, 1959), using test serum diluted one in four in the first layer.

Examination for Sjögren's Syndrome.—Each patient underwent an ophthalmic examination. This included a Schirmer I test using the standardized sterile strips developed by Halberg and Berens (Contactisol Inc. Lindenhurst, New York, U.S.A.). The wetting of the paper strip was read at 5 minutes and the mean of the two eyes was recorded. Patients with less than 15 mm. wetting were subjected to a Schirmer II test, using 10 per cent. ammonia held by the patient for 5 minutes 6 in. from the nose. Rose bengal 1 per cent. was instilled into the conjunctival sac of each eye and immediately followed by irrigation with normal sterile saline; the patients were then examined by a Zeiss or Haag-Streit slit lamp for punctate and filamentary keratitis. Staining over the area previously in contact with the Schirmer filter paper was ignored. Kerato-conjunctivitis sicca was diagnosed when the Schirmer II test gave less than 5 mm. wetting after 5 minutes and when there was associated strongly positive rose bengal staining of the conjunctivae and/or corneae. To minimize bias in this study the medical diagnosis was not known to the ophthalmologists until after the eye examinations were complete.

Sialography.—This was performed in 109 patients, 41 with Hashimoto's thyroiditis, 32 with primary hypothyroidism, and 36 control subjects. A hydrostatic technique was employed, using Triosil "45" (sodium metrizoate) as a contrast medium. Apparatus consisting of a 20 ml. glass syringe, polythene tubing, and a tapered catheter was used to convey the contrast medium to the duct and gland. The glass syringe was set at a fixed height above the patient's head (70–90 cm.). The contrast medium flowed into the gland using only the force of gravity and therefore a relatively constant pressure was obtained (Park and Mason, 1966). This method rarely led to over-filling and was less painful for the patient. The water-soluble contrast medium is rapidly expelled from the gland and therefore a secretory phase film was exposed 5 minutes after the filling phase was complete. Between the two phases, the patient was asked to suck a slice of lemon to stimulate salivary flow.

Results

The results of the ophthalmological examination are summarized in Table I. Only a small minority in each group had definite kerato-conjunctivitis sicca. The highest prevalence was found in Group 3 treated thyrotoxicosis (8·6 per cent.), but this was not significantly different from that in Group 5 hospital controls (6·9 per cent.). The 43 patients treated with ^{131}I therapy were re-assessed separately (Table II, overleaf). Six of those with kerato-conjunctivitis sicca came from this group and none of the remaining 25 treated by drugs and/or surgery had kerato-conjunctivitis sicca. No correlation was, however, found between the presence of kerato-conjunctivitis sicca and the number of doses, total dosage of ^{131}I, or the interval since the last dose of ^{131}I.

None of the 83 patients with Hashimoto's thyroiditis and none of the four with primary hypothyroidism plus rheumatoid arthritis had kerato-conjunctivitis sicca.

TABLE II

RELATION OF KERATO-CONJUNCTIVITIS SICCA TO THE NUMBER OF DOSES AND TOTAL DOSAGE OF RADIOACTIVE IODINE (^{131}I) THERAPY IN THYROTOXIC PATIENTS

No. of Patients	No. of Doses of ^{131}I	Mean and Range of ^{131}I Doses (mc.)		No. with Kerato-conjunctivitis Sicca
		Mean	Range	
15	1	9·1	8–10	2
18	2	18·7	16–20	3
10	3	29·1	24–30	1

None of the five patients with Hashimoto's thyroiditis in whom the diagnosis was confirmed by biopsy had kerato-conjunctivitis sicca.

Abnormal sialograms were found in eighteen cases: seven (16 per cent.) of the 41 patients with Hashimoto's thyroiditis, five (16 per cent.) of the 32 with primary hypothyroidism, and in six (16 per cent.) of the 36 in the hospital control group (Table III). Only two patients with Hashimoto's thyroiditis showed globular sialectasis, the remaining patients having only minor abnormalities consisting of punctate sialectasis with or without intermediate duct changes. Mild xerostomia was found in sixteen of these eighteen patients. None had a history or clinical evidence of salivary gland enlargement. Two of the hospital control subjects had unexplained xerostomia but normal sialograms. None of the patients with Hashimoto's thyroiditis or with primary myxoedema who had rheumatoid arthritis had xerostomia or abnormal sialograms.

TABLE III

RESULTS OF SIALOGRAPHY IN CASES OF HASHIMOTO'S THYROIDITIS AND PRIMARY HYPOTHYROIDISM, AND IN HOSPITAL "CONTROLS"

Clinical Group	No. of Patients	Sialographic Abnormalities					No. with Abnormal Sialograms and Xerostomia	No. with Abnormal Sialograms and Salivary Gland Enlargement
		Total		Punctate	Punctate with Intermediate Duct Changes	Globular		
		No.	Per cent.					
Hashimoto's Thyroiditis	41	7	16	3	2	2	6	0
Primary Hypothyroidism	32	5	16	3	2		4	0
Hospital Controls	36	6	16	4	2		6	0

Discussion

This study shows no increased prevalence of kerato-conjunctivitis sicca in patients with proven autoimmune thyroid disease (Table I). The prevalence is higher than that in an ophthalmic control series of 6,200 (0·2 per cent.) in the United States (de Roetth, 1945), but the age and sex distribution of de Roetth's patients was not recorded. The number of patients with xerostomia and sialographic abnormalities consistent with Sjögren's disease affecting the parotid glands was also no higher in the groups with Hashimoto's thyroiditis and primary hypothyroidism respectively than in the hospital control group (Table III).

The prevalence of kerato-conjunctivitis sicca in patients with thyrotoxicosis (8·6 per cent.) was higher, but not significantly higher, than in the patients with simple goitre (4·3 per cent.) and the hospital controls (6·9 per cent.). 64 per cent. of

the patients with thyrotoxicosis received treatment with ^{131}I therapy, but no correlation was found between the number of doses or the total amount of ^{131}I given. One of us (Williamson, unpublished observations) has detected significant amounts of ^{131}I in tears one hour after a therapeutic dose of ^{131}I given to thyrotoxic patients. It does not appear, however, that this results in subsequent irradiation damage to the lacrimal and accessory lacrimal glands of the eye. It is of interest to note, however, that irradiation parotitis and xerostomia have been noted in patients receiving similar doses of ^{131}I therapy for thyrotoxicosis (Chapman and Maloof, 1955).

The prevalence of thyroglobulin autoantibodies in patients with Sjögren's syndrome is, however, increased (Anderson and others, 1961; Bunim, 1961; Bloch and Bunim, 1963; Anderson and others, 1965), and thyroglobulin autoantibodies have also been reported to occur with increased frequency in the connective tissue diseases, rheumatoid arthritis (Anderson and others, 1961; Bloch and others, 1965), and systemic lupus erythematosus (Anderson and others, 1961; Hijmans, Doniach, Roitt, and Holborow, 1961), all of which may be associated with established kerato-conjunctivitis sicca. The absence of an increased prevalence of kerato-conjunctivitis sicca in autoimmune thyroid disorders in contrast to that in autoimmune systemic disorders may, however, be consistent with the concept that autoimmune thyroiditis is an organ specific disorder.

Summary

83 patients with Hashimoto's thyroiditis, 69 with primary hypothyroidism, and 68 with thyrotoxicosis were examined for kerato-conjunctivitis sicca by Schirmer tear tests, staining of the conjunctiva and cornea by rose bengal dye, and slit-lamp examination. The prevalence of kerato-conjunctivitis sicca in these patients was no higher than in 46 patients with simple goitre and in 72 hospital controls matched for age and sex. Sialography was performed in 41 patients with Hashimoto's thyroiditis, 23 with primary hypothyroidism, and 36 of the hospital controls. Sialographic abnormalities suggestive of Sjögren's syndrome were found as frequently in the hospital controls as in the patients with Hashimoto's thyroiditis and primary hypothyroidism.

We are greatly indebted to Prof. E. M. McGirr, University Department of Medicine, Royal Infirmary, Glasgow, for allowing us to examine patients attending his clinics.

Dr. W. Watson Buchanan, Centre for Rheumatic Diseases, Baird Street, Glasgow, gave invaluable direction and advice in organizing and conducting this survey; without his help we would have been unable to complete our task.

Dr. Robert Goudie and his colleagues, Western Infirmary, Glasgow, carried out the serological studies for autoimmune antibodies. Finally, we should like to thank Dr. W. Wilson, Ophthalmic Institute, Glasgow, for editing the drafts.

A grant from the Arthritis and Rheumatism Council for Research supported most of this work.

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Printed in Great Britain by J. Smethurst & Co. Ltd., 35 Rothschild Road, London, W.4.

35

FUNGAL FLORA OF THE CONJUNCTIVAL SAC IN HEALTH AND DISEASE

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Reprinted from British Journal of Ophthalmology
Volume 52, *No.* 2, *pages* 127–137, *February*, 1968

LONDON
BRITISH MEDICAL ASSOCIATION
TAVISTOCK SQUARE, W.C.1

Brit. J. Ophthal. (1968) 52, 127

FUNGAL FLORA OF THE CONJUNCTIVAL SAC IN HEALTH AND DISEASE*†

INFLUENCE OF TOPICAL AND SYSTEMIC STEROIDS

BY

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ALTHOUGH the role of fungi as ocular pathogens has become more widely recognized (Maddren, 1941; Sykes, 1946; Mendelblatt, 1953; Mitsui and Hanabusa, 1955; Roberts, 1957; Haggerty and Zimmerman, 1958; Mikami and Stemmermann, 1958; Fine and Zimmerman, 1959; Chick and Conant, 1962; Currie, 1963; Ainley and Smith, 1965; Jelenkiewicz, 1965; Dominiczak, Branicka, and Lewenstam, 1965), relatively little attention has been given to the fungal flora of the healthy or diseased eye. Previous lengthy investigations conducted in Europe (Fazakas, 1935, 1953) and in the U.S.A. (Hammeke and Ellis, 1960) have shown some disparity in the frequency of occurrence of fungi in the healthy conjunctival sac; studies of smaller groups of patients (Mitsui and Hanabusa, 1955; Azevedo, 1962) are less conclusive owing to the small size and selectivity of the samples. Furthermore, some ocular mycoses may have an exogenous source, especially after surgery or trauma (Fine and Zimmerman, 1959) and an accurate knowledge of the species most likely to be encountered in the conjunctival sac is of more than academic interest. The first purpose of this paper, therefore, is to report the results of an investigation of the fungal flora of the clinically healthy conjunctivae of a large and representative sample of subjects in various age groups.

Our second purpose is to study the variations of fungal flora in corticosteroid-treated patients. It is well documented that steroids reduce tissue resistance to a wide variety of bacterial, viral, and fungal agents (Zimmerman, 1950; Kligman, Baldridge, Rebell, and Pillsbury, 1951; Selye, 1951), and it would appear that the increase of ocular mycoses within recent years may be associated with the extensive use of systemic and topical corticosteroids and of broad-spectrum antibiotics (Hogan, Thygeson, and Kimura, 1954; Suie and Havener, 1963; McLean, 1963). Consequently, we have investigated the effects of (*a*) topical steroids, (*b*) topical steroids combined with antibiotics, and (*c*) systemic steroids, on the conjunctival flora of various groups of patients.

The third section is concerned with the fungal flora of the eye in Sjögren's syndrome. The characteristically dry eye encountered in this syndrome (keratoconjunctivitis sicca) is associated with depletion of normal tear flow and impaired mechanical removal of foreign material, and may therefore be more susceptible to colonisation by microbial agents.

Material and Methods

Patients

(1) *Clinically Healthy Conjunctivae*

553 patients comprising 284 males and 269 females (1,106 eyes), without clinical evidence of

* Received for publication February 1, 1967.
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external ocular inflammation, were chosen for the investigation of the fungal flora of the healthy conjunctival sac. Each decade of age was represented by at least forty patients, equally divided between the sexes (Table I). The children in the 0 to 9 year age group were obtained mainly from an orthoptic department; the 10 to 19-year-olds from an ophthalmic out-patient department and from a mass radiography centre.

TABLE I
PATIENTS WITH CLINICALLY NORMAL CONJUNCTIVAE, BY AGE GROUP AND SEX

Age Group (yrs)		0–9	10–19	20–29	30–39	40–49	50–59	60–69	70+	Total
Sex	Male	39	105	21	20	27	28	34	20	284
	Female	28	104	23	22	22	25	23	22	269
Total Patients		57	209	44	42	49	53	57	42	553

The association of conjunctival fungi with topical steroid therapy was examined in two series of patients.

(2) *Clinically Healthy Conjunctivae subjected to Topical Therapy*

Adult patients with no clinical evidence of external ocular disease were selected from an ophthalmic out-patient department and from a centre for rheumatic diseases.

(*a*) Cultures were taken before and after a course of betamethasone disodium phosphate (0·1 per cent. in water-miscible base) in 86 patients (26 males and 60 females; 165 eyes). The age range was 30 to 78 years (mean 62). 36 patients had various forms of cataract, 35 had refractive errors, and fifteen had glaucoma.

(*b*) Cultures were taken from 52 patients (17 males and 35 females; 104 eyes) before and after a course of betamethasone disodium phosphate (0·1 per cent.) and neomycin sulphate B.P. (0·5 per cent.) in water-miscible base) (Betnesol-N, Glaxo) applied three times daily for 1 week. The age range was 28 to 75 years (mean 60·5). 25 of the patients in this group had various forms of cataract, three refractive errors, 23 rheumatoid arthritis, and one systemic lupus erythematosus. Altogether 138 patients (269 eyes) with clinically healthy conjunctivae were thus "treated" with the topical steroid or with the steroid/antibiotic drops for one week, the fungal flora of the conjunctival sac being determined before commencement and after the completion of treatment.

(3) *Diseased External Eyes subjected to Topical Steroid Therapy*

The second main clinical category comprised patients with various forms of external ocular disease, for which either betamethasone disodium phosphate or betamethasone disodium phosphate combined with neomycin sulphate had been prescribed.

(*a*) 46 patients (10 males and 36 females; 74 eyes; age range 15 to 58 years, mean 53) had been receiving the steroid preparation. Table II shows the conditions being treated, and Table III the duration of steroid treatment. Mydriatics were being instilled into nine eyes, miotics into three, and methyl cellulose drops into one eye.

TABLE II
OCULAR DISEASE

Disease under Treatment	No. of Patients	No. of Eyes
Conjunctivitis	15	25
Blepharo-Conjunctivitis	10	20
Episcleritis	5	8
Anterior Uveitis	15	20
Scleritis	1	1
Total	46	74

TABLE III
DURATION OF TREATMENT

Duration	No. of Eyes
1–4 wks	30
1–12 mths	40
1–3 yrs	4
Total	74

(*b*) 55 patients (10 males and 45 females; 75 eyes; age range 23 to 61 years, mean 54·5) had been receiving the steroid/antibiotic drops. The conditions under treatment are shown in Table IV and the duration of therapy in Table V. Mydriatics were being instilled into six of these eyes.

TABLE IV
OCULAR DISEASE

Disease under Treatment	No. of Patients	No. of Eyes
Conjunctivitis	16	21
Blepharo-Conjunctivitis	15	25
Episcleritis	1	1
Keratitis	15	19
Anterior Uveitis	4	4
Styes	4	5
Total	55	75

TABLE V
DURATION OF TREATMENT

Duration	No. of Eyes
1–4 wks	30
1–12 mths	38
1–3 yrs	7
Total	75

(4) *Conjunctivae of Patients receiving Systemic Steroid Therapy*

The association of conjunctival fungi with systemic steroid therapy was examined in thirty randomly chosen ward patients who were being treated in a centre for rheumatic diseases.

There were eight males and 22 females; age range 18 to 57 years (mean 52). Two of the patients had a probable recurrence of rheumatic fever and the remainder had rheumatoid arthritis. The duration of therapy varied from 1 month to more than 3 years, and the daily prednisolone intake from 5 to more than 16 mg. The total dosage of steroid ranged from 1·5 to over 9 g.

(5) *Conjunctivae of Patients with Sjögren's Syndrome*

The last clinical group chosen for the investigation of the fungal flora of the conjunctival sac consisted of 37 patients (3 males and 34 females; 74 eyes; age range 37 to 80 years, mean 55·2) with severe keratoconjunctivitis sicca attending a special "dry-eye" clinic. The clinical diagnosis was based on a history of dry, irritable, or itchy eyes, a strongly positive Schirmer II tear test (less than 5 mm.) using 10 per cent. ammonia, and the presence of marked staining of both conjunctiva and cornea with 1 per cent. rose bengal. In addition, all of these patients had rheumatoid arthritis based on American Rheumatism Association criteria (Ropes, Bennett, Cobb, Jacox, and Jessar, 1958), and they were all attending a centre for rheumatic diseases. Fourteen of the subjects were receiving systemic prednisolone (5–15 mg. daily), but none was receiving topical steroids or antibiotics. Topical treatment was discontinued for 3 to 4 days before the initial culture in order to allow as much discharge as possible to collect.

Collection of Specimens

In all cases, specimens for culture were taken from both conjunctival sacs using a stiff nickel-chrome wire loop, with which the lower fornices were vigorously scraped. In the case of the keratoconjunctivitis sicca patients, any ropy discharge was also utilized for culture. Any cultures found to be positive were repeated. Two hundred blank tubes, inoculated in the clinics under similar working conditions, were submitted to the laboratory interspersed in irregular batches with the clinical samples. Cultures were also taken at irregular intervals from the mydriatics, miotics, vital dyes, and methyl cellulose drops in current use at the ophthalmic out-patient department.

Cultivation of Yeasts and Fungi

The mycological growth medium employed throughout this investigation was 2 per cent. malt extract agar, containing 0·036 per cent. potassium tellurite as a bacterial inhibitor. The medium was dispensed as drops in cotton-wool-plugged sugar tubes. After inoculation and transmission to the laboratory, all tubes (including the blanks) were incubated at 25°C., and were examined for evidence of fungal growth at intervals of 7 days. Negative cultures were reincubated and no tube was discarded before the end of 4 weeks' incubation.

Yeasts and yeast-like fungi were identified according to the taxonomic descriptions of Lodder

and Kreger-Van Rij (1952). In particular, the specific identification of *Candida* species was based upon the results of sugar fermentation and carbohydrate assimilation tests (Lodder and Kreger-Van Rij, 1952). The identity of *Candida albicans* was confirmed by the demonstration of characteristic germ tubes in human serum, as described by Taschdjian, Burchall, and Kozinn (1960), and by the demonstration of chlamydospore formation on corn meal agar slide cultures. The identification of the filamentous fungi was based upon their gross colonial morphology on malt agar, and upon the nature and arrangement of their spores on malt agar slide cultures. The technique of slide culture was as described by Riddell (1950). In order to reduce bias during the study, the bacteriologist was not informed of the sources of the cultures.

Results

Table VI shows the frequency and generic identity of the fungal isolates from 1,106 healthy conjunctival sacs in various age groups. The overall incidence of fungi was 2·9 per cent., the lowest frequency (0·8 per cent.) being observed in the 0 to 9 year age group, and the highest (7 per cent.) in the 60 to 69-year age group. Although there were more isolates from patients over than from those under the age of 40 years, a progressive increase of incidence of isolates with increasing age was not demonstrated.

TABLE VI
ANALYSIS OF POSITIVE FUNGAL CULTURES FROM HEALTHY CONJUNCTIVAE, BY AGE GROUP

Age Group (yrs)	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70+	Total
Penicillium Sp.	–	–	1	1	3	1	1	–	7
Aspergillus Sp.	–	1	–	–	–	3	–	–	4
Rhodotorula Sp.	–	–	2	–	–	–	1	–	3
Scopulariopsis Sp.	–	–	1	–	–	1	–	1	3
Candida Sp.	–	2	–	–	–	–	1	–	3
Isaria Sp.	1	–	–	–	–	–	1	1	3
Geotrichum Sp.	–	–	–	–	–	–	3	–	3
Papulospora Sp.	–	1	–	–	–	–	–	–	1
Gliocladium Sp.	–	–	–	–	1	–	–	–	1
Hormodendron Sp.	–	–	–	–	–	–	1	–	1
Saccharomyces Sp.	–	1	–	–	–	–	–	–	1
Rhizopus Sp.	–	1	–	–	–	–	–	–	1
Nigrospora Sp.	–	–	–	–	–	1	–	–	1
Total No.	1	6	4	1	4	6	8	2	32
Per cent.	0·8	1·4	4·6	1·2	4	5·6	7	2·4	2·9

A comparison of the fungal flora of clinically normal external eyes before and after the topical application of a steroid or steroid/antibiotic preparation for one week is shown in Table VII (opposite). No significant increase in the frequency of isolation of fungi from the eyes of 86 patients treated with betamethasone disodium phosphate was observed, but the application of the betamethasone disodium phosphate/neomycin sulphate preparation was associated, over the same period of time, with a significant increase in incidence of fungi in the conjunctival sacs of 52 patients (χ^2 using Yates correction for continuity for small numbers = 8·2174; $P < 0·01$). On the other hand (Table VIII, opposite), the conjunctival sacs of patients with external ocular diseases being treated with the steroid or steroid/antibiotic preparation did not yield fungi any more frequently than the healthy sacs. There was no significant difference in isolation rates between patients subjected to topical steroids alone and those subjected to topical steroids combined with an antibiotic. Furthermore, repeats of the initially positive fungal cultures were uniformly negative, suggesting

TABLE VII

PATIENTS WITH CLINICALLY NORMAL EXTERNAL EYES GIVEN A TOPICAL STEROID OR STEROID/ANTIBIOTIC PREPARATION

Treatment Group	No. of Patients	Culture			
		First		Second	
		No. Positive	Organism	No. Positive	Organism
Betamethasone Disodium Phosphate	86 (165 eyes)	2+ve	*Aspergillus* *Rhodotorula*	3	*Aspergillus* *Penicillium* *Saccharomyces*
Betamethasone Disodium Phosphate/ Neomycin Sulphate	52 (104 eyes)	1+ve	*Candida albicans*	11	3 *Candida albicans* 3 *Penicillium* 3 *Aspergillus* 1 *Streptomyces* 1 *Trichoderma*

TABLE VIII

PATIENTS WITH EXTERNAL OCULAR DISEASE RECEIVING A TOPICAL STEROID OR STEROID/ANTIBIOTIC PREPARATION

Treatment Group	No. of Patients	Initial Culture		Repeat of Initially Positive Cultures
		No. Positive	Organism	
Betamethasone Disodium Phosphate	46 (74 eyes)	1	*Penicillium*	Negative
Betamethasone Disodium Phosphate Neomycin Sulphate	55 (75 eyes)	4	*Penicilium* *Aspergillus* *Scopulariopsis* *Rhodotorula*	Negative

that fungal contamination was transitory. Only one positive culture was obtained from the thirty patients receiving systemic steroid therapy.

Table IX (overleaf) shows the occurrence of fungi in the conjunctival sacs of 37 patients with Sjögren's syndrome after 4 days without their usual ocular toilet. Four isolates were obtained from the fourteen patients (28 eyes) receiving systemic prednisolone, and six isolates from the remaining 23 patients (46 eyes)—an overall incidence of 13·3 per cent. This is a significantly higher incidence than that in a group of patients matched for age and sex with clinically healthy conjunctival sacs ($\chi^2 = 5{\cdot}258$; $P < 0{\cdot}05$). Further specimens taken from these patients following resumption of their usual topical therapy did not yield fungi. Eight of the ten patients in this group, who had previously harboured conjunctival fungi, were sampled once again 4 days after ceasing ocular toilet. Seven fungal isolates were obtained, four of which were species of *Candida*.

Two of the 200 blank cultures yielded aerial contaminants.

Discussion

Previous surveys of the fungal flora of the healthy conjunctival sac have been undertaken by Fazakas (1935, 1953) in Central Europe, by Mitsui and Hanabusa (1955) in Japan, by

TABLE IX
PATIENTS WITH SJÖGREN'S SYNDROME

Treatment	No. of Patients	Initial Culture		First Repeat of Positive Cultures	Second Repeat of Positive Cultures	
		No. Positive	Organism			
Systemic Steroids	14 (28 eyes)	4	*Penicillium* *Rhodotorula* *Rhodotorula* *Candida albicans*	Negative	3	Negative *Aspergillus* *Rhodotorula* *Candida albicans* Not Repeated
No Systemic Steroids	23 (46 eyes)	6	*Stemphylium* *Rhodotorula* *Candida albicans Tropicalis* *Penicillium* *Candida albicans* *Hormiscium*	Negative	4	*Candida albicans* *Penicillium* *Candida albicans Parapsilosis* *Candida albicans* Not Repeated

Hammeke and Ellis (1960) in the United States, and by Azevedo (1962) in Brazil. Ainley and Smith (1965), the only British workers who have so far undertaken a study of the fungal flora of the clinically normal conjunctiva, described a small series comprising 43 patients with no evidence of external ocular disease. The present investigation has utilized material from 1,106 healthy eyes, and is the largest series so far reported in Great Britain. In a study of 993 healthy eyes, Fazakas (1953) obtained 253 positive fungal cultures (25·4 per cent.). Hammeke and Ellis (1960) investigated 520 healthy eyes of adults, children, and neonates; 10·3 per cent. of 416 adults, 5 per cent. of 52 children, and 0·1 per cent. of 52 infants gave positive fungal isolates. Ainley and Smith (1965) studied 43 healthy eyes, and obtained twelve positive results (27·4 per cent.). We have obtained 32 positive cultures from 1,106 healthy eyes, a much lower incidence of 2·9 per cent.

Our clinical methods seem to be similar to those of other investigators and the lower incidence in this series may be partly explained by the cultural techniques employed. Thus, Ainley and Smith (1965) used Sabouraud's broth with subculture, after one week, to Sabouraud's agar plates, and subsequent incubation at 25°C. for up to 6 weeks. We used malt agar slants for primary isolation, and an incubation period extending to 4 weeks at 25°C. Hammeke and Ellis (1960) also employed Sabouraud's glucose agar, but the length of incubation of their cultures is not stated. In general, primary incubation in broth might be expected to yield a higher isolation rate than on solid medium.

Previous workers have indicated that variations in the frequency of certain fungi occur between various geographical regions, *e.g. Candida* species (Urrets-Zavalia, Remonda, and Ramacciotti, 1958) and *Sporotrichum* Sp. (Gordon, 1947; McGrath and Singer, 1952). Despite a low overall incidence of fungi, our results are in fairly good qualitative agreement with those of other investigators in other geographical areas with regard to the genera of fungi and yeasts most frequently found in the conjunctival sac. Thus, *Aspergillus* Species, *Rhodotorula*, *Candida*, and *Penicillium* Species appear to be common inhabitants of the healthy external eye (Fazakas, 1953; Mitsui and Hanabusa, 1955; Hammeke and Ellis, 1960; Ainley and Smith, 1965). These fungi collectively accounted for 54 per cent. of the total isolates in our series. Fazakas (1953) found that the majority of his isolates from healthy eyes were moulds, 28 per cent. of the isolates in his series of 993 eyes belonging to

the *Penicillium* group. 22 per cent. of the positive cultures in our "healthy eye" group were *Penicillium*.

In contrast to the findings of Hammeke and Ellis (1960), who reported distinct differences in the frequency of positive fungal isolates from conjunctival sacs in different age groups, we have not observed a progressive increase in incidence of fungi with increasing age, although the overall incidence of fungi in the older age groups was somewhat higher than in the very young age groups.

One may question the significance of the presence of fungi in the healthy conjunctival sac. In an attempt to provide a partial answer to this question, we repeated all of our 32 positive cultures within 4 weeks of their initial detection, but we obtained only four repeat positives. Furthermore, in none of these cases was the same species recovered. On the basis of these findings, we consider that the fungi cultivable from healthy conjunctival sacs must be regarded as transitory contaminants rather than resident commensals. Although it would appear, therefore, that little significance should normally be attached to the presence of fungi in the conjunctival sac, we nevertheless consider that it is important to have accurate knowledge of the fungal species most likely to be encountered there, even temporarily. The role of fungi as pathogens in ocular infections is becoming more widely recognized, and although an increased awareness of the possibility of ocular mycoses may be partly responsible, there is now well-documented evidence of a real increase in the incidence of mycotic infections of the eye (Haggerty and Zimmerman, 1958; Mikami and Stemmermann, 1958; Fine and Zimmerman, 1959; Chick and Conant, 1962), especially as a sequel to trauma or surgery of the eye (Fine and Zimmerman, 1959), where infection is believed to be exogenous in origin. In the latter instance, conjunctival saprophytes might assume a pathogenic role in an "opportunistic" infection, as recently discussed in the more general sphere of microbial disease by Symmers (1965).

The effect of corticosteroids in reducing resistance to a variety of bacterial, viral, and fungal infections is well recognized (Zimmerman, 1950; Kligman and others, 1951; Selye, 1951; Symmers, 1965), and it is believed that corticosteroids may permit fungi, normally regarded as harmless commensals, to behave as pathogens (Agarwal, Malik, Mohan, and Khosla, 1963; Suie and Havener, 1963). There is evidence that the extensive systemic and topical use of corticosteroids and broad-spectrum antibiotics has largely contributed to the increase of ocular mycoses (Hogan and others, 1954; Suie and Havener, 1963; McLean, 1963; Manchester and Georg, 1959; Wolter, 1962; Currie, 1963). There is also clear experimental evidence for the enhancement of the effects of fungus infection by corticosteroids (Mankowski and Littleton, 1954; Ley, 1956; Hirose, Yoshioka, Abe, Kanemitsu, and Kiya, 1957; Agarwal and others, 1963). Although such experimental conditions may have little counterpart in human ocular infections, there are frequent reports at the clinical level of ocular mycoses complicating steroid therapy, especially in relation to *Candida* species. Sykes (1946), Mendelblatt (1953), Mitsui and Hanabusa (1955), and Roberts (1957) have all reported corneal infections by *Candida albicans*. Maddren (1941) reported a case of severe angular conjunctivitis occurring in the course of extensive candidiasis in a woman, but Duke-Elder (1938) has stated that fungus infections of the conjunctiva are very rare. A case of ocular mycosis due to *Candida parapsilosis* was reported by Manchester and Georg (1959). Their patient was thought to have received corticosteroid and antibiotic drops for a long period before developing keratomycosis, the initial lesion being a superficial punctate keratitis. Currie (1963) described three cases of mycotic keratitis associated

with corneal ulceration. *Candida albicans* was implicated, and he considered that steroids had aggravated the condition. Ainley and Smith (1965) have more recently described a probable case of secondary keratomycosis due to *Candida parapsilosis* which responded to the administration of Nystatin.

It is of interest that both *Candida albicans* and *Candida parapsilosis* were recovered in our series of clinically normal conjunctivae, and that *Candida albicans* was represented in our isolates from patients receiving the topical steroid/antibiotic preparation.

Previous studies have been undertaken of the effects of corticosteroid therapy upon the incidence of fungi in the eye. Mitsui and Hanabusa (1955) obtained 42 positive cultures from 62 patients receiving topical ocular steroids (67 per cent.), while a control group of untreated patients had an incidence of 18·5 per cent. The majority of the isolates in their series were *Penicillium*, *Aspergillus*, *Candida*, *Saccharomyces*, and *Rhodotorula* species. In a second experiment, these authors selected eighteen cases initially negative for fungi by smear or culture. After topical application of hydrocortisone ointment for 3 weeks, the eyes of nine of the subjects were positive for fungi, *Penicillium* and *Rhodotorula* species predominating. Ainley and Smith (1965) failed to demonstrate any striking change in fungal flora after the application of a corticosteroid/antibiotic combination (Betnesol-N) to the eyes. Thus, of fifteen patients initially showing negative cultures, only three became positive for fungi after the administration of drops or ointment thrice daily for not less than 2 weeks. They point out that the number of patients studied was too small to give a statistically significant result.

In the present investigation, topical betamethasone treatment of patients with clinically normal external eyes and of patients with external ocular disease, did not result in any significant changes in the mycotic flora of the eye. Thus, 86 patients (165 eyes) with clinically normal external eyes received betamethasone disodium phosphate three times daily for 1 week; only two pre-treatment isolates and three post-treatment isolates were obtained. Furthermore, no generally accepted fungal pathogens were represented. 46 patients (74 eyes) with external ocular disease, who had been receiving topical betamethasone disodium phosphate for varying periods of time, yielded one fungal isolate, a saprophytic *Penicillium* species, and a further conjunctival sac scraping from the same patient was negative.

Four fungal isolates of no pathogenic significance were obtained from the eyes of 55 patients (75 eyes) with various forms of external ocular inflammation, who had been receiving the betamethasone disodium phosphate/neomycin sulphate preparation for varying periods; repeat cultures were uniformly negative. The combined steroid/antibiotic preparation, therefore, produced no significant changes in fungal flora in this group.

The effects of the topical administration of the betamethasone/neomycin preparation to the patients with clinically normal external eyes deserve comment. Thus, there was one isolate from 104 eyes before the commencement of therapy, and eleven isolates, including three strains of *Candida albicans* after its completion. It is difficult to offer a simple explanation of the comparatively large increase in ocular fungi in this particular group of subjects, but it is of interest that almost 50 per cent. of the patients in this category were hospital in-patients (23 rheumatoid arthritis, 1 systemic lupus erythematosus). When the cultural results are considered in relation to the source of the patient, ten of the eleven post-treatment isolates were derived from the 24 ward patients, whereas only one post-treatment isolate was obtained from the remaining eighteen out-patients. The higher incidence of

fungi in the ward patients might possibly reflect a high level of aerial fungal contamination of the ward environment when the specimens were collected. On the other hand, only one fungal species was recovered from thirty in-patients with rheumatoid arthritis receiving systemic steroids in the same wards as the former group.

There is no evidence from the present investigation that the neomycin component of the combined topical steroid/antibiotic preparation made any significant contribution to the alteration of fungal flora in patients so treated. Thus, there was no significant difference in the frequency of isolation of fungi from the eyes of patients with external ocular disease, whether treated with steroid/antibiotic combination or with steroid alone (Table VIII). It is, however, relevant to note that numerous workers have shown that antibiotics, especially the tetracyclines, can enhance the growth of fungi, notably *Candida albicans*, and that subjects treated with antibiotics are more often carriers of *Candida albicans* than are untreated controls (McGovern, Parrott, Emmons, Ross, Burke, and Rice, 1953; Sharp, 1954). There are relatively few reports to incriminate neomycin in this respect, though Reiersöl (1958) observed a marked increase in the incidence of faecal *Candida albicans* in patients given oral neomycin, and it is possible that local neomycin therapy might give rise to a similar increase in the conjunctiva.

The group of patients with Sjögren's syndrome yielded some interesting results. Ten primary isolates were obtained from 74 eyes. The patients were derived from a "dry-eye" clinic and they were instructed to use no local treatment, not even saline washouts, for 4 days before the first culture. Thereafter, they were allowed to return to their usual routine of thrice daily irrigations with saline and instillations of carboxymethyl-cellulose drops (0·5 per cent. solution) twice or three times daily. The second cultures were taken while the patients were receiving this irrigation regime, and the totally negative cultural results could be adequately explained on the basis of mechanical removal of foreign material from the conjunctival sacs by the irrigations. This thesis is supported by the fact that seven fungal isolates were subsequently obtained from the eyes of eight patients who consented to stop all local treatment for 4 days before the collection of further specimens. Any fungistatic effect of the carboxymethyl-cellulose drops (which contains chlorhexidine digluconate, 0·05 per cent.) was excluded by failure to demonstrate inhibition of twenty strains of *Candida albicans*, three strains of *Rhodotorula*, and two *Aspergillus* strains, in simple plate diffusion tests in agar.

Patients receiving systemic steroids and those on alternative treatment showed no significant differences in the frequency of isolation of fungi, but the results suggest that the dry eye accompanying Sjögren's syndrome is more susceptible to colonization with fungi than the healthy eye. Furthermore, patients with Sjögren's syndrome tend to belong to an ageing group, and intra-ocular surgery for cataract extraction or glaucoma may become a necessity. In view of the more frequent isolation of fungi from these patients, including the potential intra-ocular pathogens *Candida albicans* and *Candida parapsilosis*, it is considered that special pre-operative precautions are necessary, particularly since fungi are sometimes thought to be introduced as a result of surgical or accidental trauma (Fine and Zimmerman, 1959).

Summary

Studies were undertaken of the fungal flora of the healthy and diseased conjunctival sac and of the effects of steroids.

Fungi were isolated from 2·9 per cent. of 1,106 healthy conjunctival sacs, a higher incidence being observed in older age groups. Although the majority of the species isolated were non-pathogenic transient aerial contaminants, some potential intra-ocular pathogens were also represented.

The effects were studied of the topical administration of betamethasone and of a combined betamethasone/neomycin preparation to patients with clinically normal external eyes. Topical betamethasone therapy did not result in any significant changes in the fungal flora of the conjunctival sac: a significantly higher incidence of fungi was observed in the eyes of hospital in-patients receiving betamethasone/neomycin treatment.

The conjunctival sacs of out-patients with a variety of external ocular diseases being treated with betamethasone or betamethasone combined with neomycin did not yield fungi any more frequently than healthy conjunctival sacs: there was no significant difference in isolation rates between patients subjected to the topical steroid alone, and those subjected to the combined steroid and antibiotic preparation.

Fungi were obtained from 13·3 per cent. of the conjunctival sacs of 37 patients with Sjögren's syndrome. This was a significantly higher incidence than that obtained from a group of patients with healthy eyes matched for age and sex. The isolates included the potential intra-ocular pathogens *Candida albicans* and *Candida parapsilosis*, and evidence was obtained that the untreated dry eye associated with this syndrome was more susceptible to fungal colonisation than the healthy eye. The possible implications of this finding were discussed.

We should like to acknowledge the continued guidance of Dr. W. Wilson, consultant ophthalmologist, Ophthalmic Institute, Glasgow, and of Dr. J. C. Gentles, medical mycologist, Glasgow University Medical Mycology Unit. Dr. J. C. J. Ives (consultant in charge, University Department of Bacteriology, Glasgow Royal Infirmary), Dr. R. Leishman (consultant ophthalmologist, Victoria and Southern General Hospitals, Glasgow), and Dr. W. W. Buchanan (consultant physician, Centre for Rheumatic Diseases and Glasgow Royal Infirmary) offered helpful criticism. Mr. K. M. Dyer (headmaster), and the staff of Onslow Secondary School, Glasgow, co-operated in the collection of specimens from the eyes of schoolchildren. The Director of the Mass Radiography Centre, Elmbank Street, Glasgow, provided the facilities for the collection of scrapings from a large number of subjects with healthy conjunctival sacs.

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SJÖGREN'S SYNDROME IN RELATION TO PERNICIOUS ANAEMIA AND IDIOPATHIC ADDISON'S DISEASE

BY

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Reprinted from British Journal of Ophthalmology
Volume 54, *No.* 1, *pages* 31–36, *January*, 1970

LONDON
BRITISH MEDICAL ASSOCIATION
TAVISTOCK SQUARE, W.C.1

Brit. J. Ophthal. (1970) **54**, 31

Sjögren's syndrome in relation to pernicious anaemia and idiopathic Addison's disease

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Lymphocytic infiltration of the gastric mucosa in chronic gastritis is associated with the development of pernicious anaemia in a proportion of cases (Anderson, Buchanan, and Goudie, 1967). More than 40 per cent. of patients suffering from pernicious anaemia can be shown to have antibodies to gastric parietal cells (Irvine, Davies, Delamore, and Williams, 1962; Markson and Moore, 1962; Taylor, Roitt, Doniach, Couchman, and Shapland, 1962; Irvine, 1963a). It occurred to the investigators that the lymphocytic infiltration in chronic gastritis might be similar to that found in the lacrimal and salivary glands in Sjögren's syndrome and it is to be noted that a series of patients suffering from this disease was found to have a high incidence of autoantibodies to gastric parietal cells (Buchanan, Cox, Harden, Glen, Anderson, and Gray, 1966). Furthermore, patients suffering from pernicious anaemia have a high incidence of thyroglobulin antibodies (Irvine and others, 1962; Markson and Moore, 1962; Taylor and others, 1962; Doniach, Roitt, and Taylor, 1963) as do patients with Sjögren's syndrome (Anderson, Goudie, Gray, and Buchanan, 1961; Bloch, Buchanan, Wohl, and Bunim, 1965).

Sjögren's syndrome consists of the triad of keratoconjunctivitis sicca, xerostomia, and rheumatoid arthritis (Sjögren, 1933) or other connective tissue diseases such as systemic lupus erythematosus (Ramage and Kinnear, 1956; Bain, 1960), polyarteritis nodosa (Ramage and Kinnear, 1956), progressive systemic sclerosis (Ramage and Kinnear, 1956), and polymyositis (Bloch and others, 1965).

The first purpose of this investigation was to determine the prevalence of Sjögren's syndrome in patients suffering from proven pernicious anaemia.

In idiopathic Addison's disease there is atrophy of both adrenal cortices with loss of most of the cortical cells, lymphocytic and plasma cell infiltration, and minimal fibrosis. Chronic thyroiditis, which demonstrates similar histological changes, is present in approximately 50 per cent. of patients with idiopathic Addison's disease examined *post mortem* (Wells, 1930; Sloper, 1953; Bloodworth, Kirkendall, and Carr, 1954). Primary myxoedema has occurred with idiopathic Addison's disease with sufficient frequency to warrant the term Schmidt's syndrome (Schmidt, 1926). In addition, the chronic thyroiditis found in idiopathic Addison's disease is associated with thyroid microsomal antibodies in about 30 per cent. of the patients studied (Blizzard and Kyle, 1963; Irvine,

Received for publication June 18, 1968
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1963a) and thus is probably of the autoimmune type. Pernicious anaemia has been reported in idiopathic Addison's disease (Blizzard and Kyle, 1963; Irvine, 1963b; Kra and Barile, 1964). Gastric mucosal biopsies reveal a high incidence of chronic gastritis in patients with idiopathic Addison's disease (Feyrter and Klima, 1952; Smith, Delamore, and Williams, 1961). In addition, gastric parietal cell antibodies are more prevalent in idiopathic Addison's disease than in tuberculous cases (Irvine, 1963b). There is, therefore, strong evidence, clinical, histological, and immunological, pointing to an association between idiopathic Addison's disease, chronic thyroiditis, and chronic gastritis (which predisposes to pernicious anaemia).

Antibodies to salivary duct epithelium have been reported in patients with idiopathic Addison's disease (Blizzard and Kyle, 1963) and similar antibodies have been detected in patients with Sjögren's disease (Bertram and Halberg, 1964; Halberg, Bertram, Söborg, and Nerup, 1965; MacSween, Goudie, Anderson, Armstrong, Murray, Mason, Jasani, Boyle, Buchanan, and Williamson, 1967).

The second purpose of this investigation, therefore, was to determine the prevalence of Sjögren's syndrome in patients suffering from idiopathic Addison's disease.

Material and methods

PATIENTS

169 patients (120 female, 49 male) comprising three groups (pernicious anaemia, idiopathic Addison's disease, and hospital controls) were included in the survey (the mean age and age range are shown in Table I). All of the patients were examined for evidence of keratoconjunctivitis sicca. The patients in the first two groups had been investigated as in-patients. Together with the help of the original case records and further specific examinations, evidence of rheumatoid arthritis, thyroid disease, and salivary duct atrophy was collected. Most of the hospital control patients were attending as out-patients and were not suffering from any disease known to have an autoimmune basis.

Group I Pernicious anaemia (77 patients: 40 female, 37 male)

Four of these had rheumatoid arthritis by the American Rheumatism Association criteria (Ropes, Bennett, Cobb, Jacox, and Jessar, 1958). Two had Hashimoto's thyroiditis (Buchanan, Alexander, Crooks, Koutras, Wayne, Anderson, and Goudie, 1961) and one had idiopathic Addison's disease (Anderson and others, 1967). One patient had thyrotoxicosis as well as rheumatoid arthritis and another had thyrotoxicosis.

Group II Idiopathic Addison's disease (primary adrenal atrophy) (20 patients: 15 male, 5 female)

The diagnosis was based on the exclusion of obvious causes, *e.g.* tuberculosis for extensive and irreversible destruction of the cortex of the adrenal glands (Anderson and others, 1967), and on the detection of antibodies to adrenal cortical cells (Anderson, Goudie, Gray, and Timbury, 1957; Blizzard and Kyle, 1963; Goudie, Anderson, Gray, and Whyte, 1966).

Group III Hospital controls (72 females)

These were patients attending clinics associated with the Western and Royal Infirmaries, Glasgow. None of the variety of general medical conditions from which they suffered had any known association with pernicious anaemia, thyroid disease, or autoimmune disorders.

Table I *Keratoconjunctivitis sicca in pernicious anaemia and idiopathic Addison's disease (primary adrenal atrophy)*

Clinical groups	*No. of patients*	*Age (yrs)*		*Schirmer's test (mm. at 5 minutes)*						*Kerato-conjunc-tivitis sicca*	
		Mean	*Range*	5		5–9		10–14			
				No.	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*	*No.*	*Per cent.*
Pernicious anaemia	77	62·5 ± 11·1	20–82	11	14·1	14	18·0	12	14·2	6	7·7
Addison's disease	20	36·6 ± 6	20–45	—	—	—	—	1	5·0	—	—
Hospital controls	72	51·9	31–74	5	6·9	10	13·9	6	8·3	5	6·9

METHODS

Antibodies to parietal cells

These were tested using the indirect immunofluorescence technique for the detection of gastric autoantibodies. Antigen was prepared from frozen unfixed sections of normal gastric fundal mucosa (Taylor and others, 1962).

Autoantibodies to thyroglobulin

These were tested by a precipitin test using the Ouchterlony-Elekplate technique (Anderson, Buchanan, Goudie, and Gray, 1962) and by the tanned red cell haemagglutination test using thyroglobulin-coated formalized tanned sheep red cells (Burroughs Wellcome) (Fulthorpe, Roitt, Doniach, and Couchman, 1961). Autoantibody to thyroid microsomes was measured by an immunofluorescence technique on unfixed frozen sections of thyroid slices (Holborow, Brown, Roitt, and Doniach, 1959).

Other laboratory data

Waaler-Rose, latex particle, and haemoglobin tests were recorded in the patients suffering from pernicious anaemia.

Examination for Sjögren's syndrome

Each patient underwent a complete ophthalmic examination. This included a Schirmer I tear test using standardized sterile strips developed by Halberg and Berens (Contactisol Inc., Lindenhurst, New York, U.S.A.). Patients with less than 15 mm. of wetting at 5 minutes had a Schirmer II tear test using 10 per cent. ammonia held by the patient for 5 minutes at 6 in. from the nose (Williamson, Cant, Mason, Greig, and Boyle, 1967). Keratoconjunctivitis sicca was diagnosed when the Schirmer II test gave less than 5 mm. wetting after 5 minutes and when there was strongly positive staining with the vital dye rose bengal 1 per cent., punctate or filamentary, of the conjunctivae and/or corneae.

Sialography

This was performed on four patients who gave a history of dryness of the mouth and throat and who had clinical xerostomia (Park and Mason, 1966).

Results

EXAMINATION FOR SJÖGREN'S SYNDROME

The results of the examination for keratoconjunctivitis sicca are summarized in Table I. A small number of patients in Group I (pernicious anaemia) and Group III (hospital controls) were suffering from keratoconjunctivitis sicca. Although the incidence of the ocular disease was higher in Group I than Group III the difference was not significant.

Most of the patients who demonstrated a reduced Schirmer I tear test, unprovoked by ammonia, had pernicious anaemia. The average age of the anaemia group of patients was 62·5 years, 10 years more than the hospital control patients, and this may account for the apparent fall in tear secretion in the anaemia group.

None of the patients with idiopathic Addison's disease had keratoconjunctivitis sicca.

Four of the patients with pernicious anaemia had xerostomia but normal sialograms.

Table II summarizes the principal findings in those patients who had pernicious anaemia and evidence of Sjögren's syndrome.

Table II *Summary of findings in patients with pernicious anaemia, keratoconjunctivitis sicca, and/or xerostomia*

Patient no.	*Pernicious anaemia*	*Kerato-conjunctivitis sicca*	*Xerostomia*	*Rheumatoid arthritis*	*Hashimoto's thyroiditis*	*Idiopathic Addison's disease*
1	+	+	0	+	0	0
2	+	+	+	0	+	0
3	+	+	0	0	+	+
4	+	+	+	0	0	0
5	+	+	0	0	0	0
6	+	+	0	0	0	0
7	+	0	+	0	0	0
8	+	0	+	0	0	0

The first four patients listed (Table II) had evidence of involvement of other diseases. Patient 1 had rheumatoid arthritis, patient 2 xerostomia and Hashimoto's thyroiditis, patient 3 Hashimoto's thyroiditis and idiopathic Addison's disease, and patient 4 xerostomia.

Three other patients had pernicious anaemia and rheumatoid arthritis but no evidence of keratoconjunctivitis sicca. One of the patients with pernicious anaemia and rheumatoid arthritis also suffered from thyrotoxicosis.

OTHER INVESTIGATIONS

A family history of pernicious anaemia was obtained from fourteen (18·2 per cent.) of the 77 patients suffering from this disease and a history of thyroid disorders in eight (10·4 per cent.).

There was no history of pernicious anaemia or thyroid disease in the families of those suffering from idiopathic Addison's disease.

25 (32·4 per cent.) of the 77 patients with pernicious anaemia had palpable thyroid glands. In twenty patients the glands were soft, in five firm. Two of the firm glands lay beneath operation scars, one for thyrotoxicosis, the other for Hashimoto's thyroiditis. Five of the patients with pernicious anaemia had proven thyroid disease; two had Hashimoto's thyroiditis, two thyrotoxicosis, and one primary myxoedema.

LABORATORY DATA

All of the patients with pernicious anaemia were receiving Cytamen injections, the mean current haemoglobin being 11·5 $\pm$ 2·1 g./100 ml.

Antibody to gastric parietal cells was detected in the sera of 35 (77 per cent.) of 46 patients, antibodies to thyroid microsomes in eighteen (39 per cent.) of 46 patients, and tanned red cell titres were positive in thirteen (28 per cent.) of 46 patients suffering from pernicious anaemia.

Discussion

This study shows no increased prevalance of keratoconjunctivitis sicca in patients suffering from pernicious anaemia (Table I). The prevalence is higher than in Sjögren's series (Sjögren, 1933), in which his nineteen patients were distributed among 36,000 hospital patients (0·05 per cent.), and in the ophthalmic control series of de Roetth (1945) in which he found 0·2 per cent. of 6,200 patients with keratoconjunctivitis sicca. However, in neither series was the age and sex distribution recorded. The number of patients suffering from proven autoimmune thyroid disease who develop keratoconjunctivitis sicca is also no higher than in a hospital control group (Williamson and others, 1967). In the present series, it is interesting to observe that two of the six patients with pernicious anaemia and keratoconjunctivitis sicca also had autoimmune thyroiditis—Hashimoto's disease (Table II)—and that one of them in addition had idiopathic Addison's disease. However, it is accepted that a patient with any one organ-specific disease has a higher than normal tendency to develop another organ-specific disorder (Anderson and others, 1967). In Sjögren's syndrome there are organ-specific features in that there is specific destruction of the lacrimal and salivary glands. Nevertheless, a non-organ-specific connective tissue disorder, usually rheumatoid arthritis, is present in over 50 per cent. of patients with Sjögren's syndrome (Bloch and others, 1965). In this series one of the pernicious anaemia patients who had keratoconjunctivitis sicca was also suffering from rheumatoid arthritis. Three others with rheumatoid arthritis and pernicious anaemia did not have Sjögren's syndrome.

The number of patients with xerostomia was no higher than in the hospital control group reported previously (Williamson and others, 1967).

No cases of Sjögren's syndrome were detected among the twenty patients who had a primary diagnosis of idiopathic Addison's disease. Their mean age (36·6 years) is, however, much younger than either the pernicious anaemia group or the hospital control group. One patient in the pernicious anaemia series who had idiopathic Addison's disease and keratoconjunctivitis sicca has been discussed already.

The prevalence of gastric parietal cell antibodies and thyroglobulin antibodies, however, is increased in Sjögren's syndrome (Buchanan and others, 1966; Anderson and others, 1961; Bloch and others, 1965). Both of these antibodies occur with increased frequency in pernicious anaemia (Irvine and others, 1962; Markson and Moore, 1962; Taylor and others, 1962; Irvine, 1963a; Doniach and others, 1963) and in idiopathic Addison's disease (Blizzard and Kyle. 1963; Irvine, 1963b). Thyroglobulin antibodies are more frequent in the connective tissue diseases rheumatoid arthritis (Anderson and others, 1961; Bloch and others, 1965) and systemic lupus erythematosus (Anderson and others, 1961; Hijmans, Doniach, Roitt, and Holborow, 1961), both of which may be associated with established keratoconjunctivitis sicca. The absence of an increased prevalence of keratoconjunctivitis sicca in pernicious anaemia or in idiopathic Addison's disease in contrast to that in autoimmune systemic disorders is consistent, however, with the concept that pernicious anaemia and idiopathic Addison's disease are organ-specific disorders.

Summary

77 patients with pernicious anaemia and twenty with idiopathic Addison's disease were examined for keratoconjunctivitis sicca by Schirmer tear tests, staining of the conjunctiva and cornea by rose bengal dye, and slit-lamp examination. The prevalence of keratoconjunctivitis sicca in these patients was no higher than in 72 hospital controls. Sialography was performed on four patients who had xerostomia but no abnormalities were detected.

We are greatly indebted to Prof. E. M. McGirr, University Department of Medicine, Royal Infirmary, Glasgow, to Dr. R. Hume, Consultant Physician, Southern General Hospital, Glasgow, and to Dr. I. Wang, Consultant Physician, Victoria Infirmary, Glasgow, for allowing us to examine patients attending their clinics. Dr. W. Watson Buchanan, Centre for Rheumatic Diseases, Baird Street, Glasgow, gave invaluable advice in preparing the paper and conducting the survey.

Dr. Robert Goudie and his colleagues, Western Infirmary, Glasgow, carried out the serological studies for autoimmune antibodies.

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Printed in England by Staples Printers Limited at their Kettering, Northants, establishment

OCCURRENCE OF ANTIBODY TO SALIVARY DUCT EPITHELIUM IN SJØGREN'S DISEASE, RHEUMATOID ARTHRITIS, AND OTHER ARTHRITIDES

A CLINICAL AND LABORATORY STUDY

BY

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Reprinted from

ANNALS OF THE RHEUMATIC DISEASES

Vol. 26 No. 5 September 1967

LONDON

BRITISH MEDICAL ASSOCIATION

TAVISTOCK SQUARE, W.C.1.

Ann. rheum. Dis. (1967), **26,** 402

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BY

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Sjøgren's disease, first described in 1933 (Sjøgren, 1933), consists of chronic inflammation of the lacrimal and salivary glands leading to dryness of the eyes (keratoconjunctivitis sicca) and dryness of the mouth (xerostomia); in a proportion of patients lacrimal and salivary gland enlargement may also be present (Bloch, Buchanan, Wohl, and Bunim, 1965; Talal, 1966). In 50 to 60 per cent. of patients the disease may be associated with a connective tissue disorder, usually rheumatoid arthritis, but occasionally also with polymyositis, polyarteritis nodosa, progressive systemic sclerosis (scleroderma), and systemic lupus erythematosus. The term "sicca syndrome" or "sicca complex" is applied to those cases of Sjøgren's disease not associated with rheumatoid arthritis or other connective tissue disorders.

In Sjøgren's disease, even in the absence of rheumatoid arthritis or other connective tissue disease (*i.e.* the sicca syndrome), there is hypergammaglobulinaemia and a high incidence of abnormal immunological reactions, such as antinuclear factors, rheumatoid factors, precipitating antibodies to tissue constituents, autoimmune complement-fixation tests, and passive cutaneous anaphylaxis in guinea-pigs (Jones, 1958; Stoltze, Hanlon, Pease, and Henderson, 1960; Anderson, Gray, Beck, and Kinnear, 1961; Anderson, Gray, Beck, Buchanan, and McElhinney, 1962; Bunim, Buchanan, Wertlake, Sokoloff, Bloch, Beck, and Alepa, 1964; Beck, Anderson, Bloch, Buchanan, and Bunim, 1965; Bloch and others, 1965). In addition to these non-organ specific reactions, the prevalence of low titre thyroid auto-antibodies is slightly higher than expected (Anderson, Goudie, Gray, and Buchanan, 1961; Bloch and others, 1965) and gastric parietal cell auto-antibodies with chronic atrophic gastritis show a higher prevalence, at least in patients studied in Glasgow (Buchanan, Cox, Harden, Glen, Anderson, and Gray, 1966). These serum factors indirectly favour the view that Sjøgren's disease may have an autoimmune basis.

Bertram and Halberg (1964) and Halberg, Bertram, Søborg, and Nerup (1965) reported the demonstration by immunofluorescence of antibody against salivary duct epithelium in eleven of nineteen patients with Sjøgren's disease, and they considered that the antigen might be organ specific, *i.e.* peculiar to salivary tissue. In the present paper we report the incidence of this salivary duct antibody (SDA) in groups of patients with the sicca syndrome (Sc), patients with Sjøgren's disease complicated by rheumatoid arthritis (Sj-RA), patients with rheumatoid arthritis alone (RA) and patients with various other arthritides. The presence of the antibody has been further studied in relation to a number of clinical and laboratory findings.

Materials and Methods

Patients

231 patients were studied. The clinical diagnosis, sex distribution, mean age, and age range are shown in Table I (opposite).

The diagnosis of Sjøgren's disease was based on the criteria described by Bloch and others (1965), and patients were required to show at least two of the three major components of the syndrome. The diagnosis of rheumatoid arthritis was based on the criteria of the American Rheumatism Association (Ropes, Bennett, Cobb, Jacox, and Jessar, 1958).

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TABLE I
INCIDENCE OF SALIVARY DUCT ANTIBODY IN VARIOUS CONDITIONS

Diagnosis	No. of Patients Studied	Sex		Age (yrs)		With Salivary Duct Antibody	
		Male	Female	Mean	Range	No.	Per cent.
Sicca Syndrome	13	1	12	64	53–78	2	15
Sjøgren's Disease with Rheumatoid Arthritis	17	4	13	55	29–81	11	65
Rheumatoid Arthritis	129	54	75	48	6–73	34	26
Systemic Lupus Erythematosus	4	—	4	35	20–60	1	25
Psoriatic Arthritis	9	1	8	49	20–65	—	—
Reiter's Syndrome	9	9	—	35	19–45	2	22
Ankylosing Spondylitis	1	1	—	43	—	—	—
Gout	6	4	2	58	36–83	—	—
Osteo-arthritis	43	12	31	58	23–78	—	—

Ophthalmological Examination

This was performed by the method described by Williamson, Cant, Mason, Greig, and Boyle (1967). Each patient was examined for evidence of keratoconjunctivitis sicca by one of us (J.W.) who was unaware of the clinical diagnosis. A Schirmer I tear test was carried out using standardized sterile paper strips developed by Halberg and Berens (Contactisol Inc., Lindenhurst, New York, U.S.A.) in an atmosphere between 60-70° F. with a relative humidity greater than 40 (Williamson and Allison, 1967). Wetting of the filter paper was read after 5 minutes and the mean of the two eyes recorded. Patients with wetting exceeding 15 mm. were considered normal and were not examined further. Those with wetting less than 15 mm. had a Schirmer II tear test and a Rose Bengal dye test performed. The Schirmer II test consisted of repeating the Schirmer I test while exposing the patient to a 10 per cent. solution of ammonia held 6 inches from the nose for 5 minutes. The Rose Bengal test consisted of instilling a 1 per cent. solution of the dye into the conjunctival sacs, followed immediately by irrigation with normal saline, and by examination with a Zeiss or Haag-Streit slit lamp for punctate and/or filamentary keratitis. Staining in the area previously in contact with the Schirmer paper was ignored.

A "definite" diagnosis of keratoconjunctivitis sicca was diagnosed when either the Schirmer I or Schirmer II test showed wetting less than 15 mm. but more than 5 mm., and the Rose Bengal dye test showed at the most only faint staining of the conjunctivae. Patients with a "possible" diagnosis did not have punctate and/or filamentary keratitis on slit-lamp examination.

Each patient was carefully questioned regarding a history of xerostomia and of associated oral and pharyngeal symptoms of Sjøgren's disease (Bloch and others, 1965). Salivary flow studies were performed using a modified Carlson-Crittenden cup with an outer chamber diameter of 20 mm. and an inner chamber diameter of 10 mm. Parotid saliva was collected from each patient under resting condition and after stimulation with fruit gums and lemon juice.

Many patients admitted to having a dry mouth (symptomatic xerostomia) but without experiencing insufficiency of saliva and/or difficulty in mastication, or requiring increased fluid intake. Their mouths appeared to be moist and salivary flow studies on a sample of them were within the normal range.

Sialography was performed on all the 231 patients, using the hydrostatic technique described by Park and Mason (1966). The criteria of abnormality in the sialograms were based on those described by Bloch and others (1965).

Other Clinical and Laboratory Data

In addition to the age and sex of the patient and the ophthalmological and oral examinations described, the following clinical facts were recorded:

Duration of arthritis, presence of subcutaneous nodules, functional grade, and *x*-ray classification (Steinbrocker, Traeger, and Batterman, 1949).

Laboratory investigations included:

Haemoglobin concentration, erythrocyte sedimentation rate (Westergren), white cell count, and assay of serum globulin.

Serological Methods

Salivary Duct Antibody (*SDA*).—Blocks of human submandibular gland obtained at autopsy not more than 10 hours after death were frozen on to metal chucks with CO_2 snow and 6μ sections were cut in a cryostat. The sera were applied undiluted to the unfixed section for 30 minutes at room temperature. After washing in normal saline buffered with veronal (pH 7·2) for 10 minutes, fluorescein-conjugated goat anti-human globulin serum was applied for 30 minutes. After a final 10 minutes

wash in buffered saline the sections were mounted in buffered glycerol and examined with a Gillett and Sibert conference microscope using blue light. To reduce non-specific fluorescent staining, the fluorescein-conjugated anti-human globulin serum was absorbed twice with dried rat liver powder.

Anti-nuclear Factor (ANF) was detected using the indirect fluorescence method described by Beck (1961) with rat liver as substrate. The sera were initially tested at a dilution of 1 in 16 and positive sera were then titrated in quadrupling dilutions till an end point of nuclear staining was obtained.

Anti-thyroglobulin was detected by the tanned red cell haemagglutination test described by Fulthorpe, Roitt, Doniach, and Couchman (1961), using thyroglobulin-coated formolized tanned sheep red cells (Burroughs Wellcome). The sera were initially tested at a dilution of 1 in 16 and positive sera were titrated in quadrupling dilutions.

Thyroid "Microsomal" Antibody was detected by the indirect immunofluorescence technique described by Holborow, Brown, Roitt, and Doniach (1959), using unfixed thyrotoxic thyroid tissue as substrate and with the test sera diluted 1 in 4.

Gastric Parietal Cell Antibodies were demonstrated by an indirect immunofluorescence technique (Adams, Glen, Kennedy, Mackenzie, Morrow, Anderson, Gray, and Middleton, 1964), using unfixed human gastric mucosa as substrate and testing the sera undiluted.

In the tests for SDA a highly reactive fluorescein-conjugated goat anti-human globulin provided by Dr. J. S. Beck was used, while in the other immunofluorescent tests commercially available fluorescein-conjugated rabbit anti-human globulin (Burroughs Wellcome) was used.

Rheumatoid Factor was determined by the Hyland latex (RA) test technique (Hyland Laboratories, California). All sera were screened at a dilution of 1 in 32 and the presence of agglutination was recorded 15 and 45 seconds after mixing the reagents. Agglutination at either 15 or 45 seconds was recorded as positive. Positive sera were then titrated in doubling dilutions.

Non-specific Tissue Precipitin Tests were performed, using the method described by Anderson, Gray, and others (1961) with human thyroid tissue as antigen. All specimens were tested undiluted and at a dilution of 1 in 8.

Results

Fig. 1 (opposite) shows positive and negative staining of salivary duct epithelium. Positive immunofluorescent staining varied in intensity, but even with the brightest staining pattern it was found that the antibody was present in low titre, none exceeding 1 in 32.

In the following statistical analysis χ^2 has been calculated (when appropriate) using Yates's correction for small numbers. Comparisons which do not yield statistically significant differences are not discussed.

Whole Series: Incidence of SDA in Various Conditions (Table I, see p. 403).

In patients with Sc, the antibody was found in only two of thirteen (15 per cent.). In contrast, the antibody was present in eleven of seventeen patients (65 per cent.) with Sj RA. In the RA group 34 of 129 patients (26 per cent.) had SDA in their serum, an incidence not differing significantly from that found in the Sc group, but lower than that in the Sj-RA group ($\chi^2 = 12{\cdot}23$; $P<0{\cdot}001$).

Of the various other groups examined, one of the four patients with systemic lupus erythematosus and two of nine males with Reiter's syndrome had SDA.

The patient with systemic lupus had definite keratoconjunctivitis sicca, severe xerostomia with objective evidence of reduced salivary flow rate, punctate sialectasis and intermediate duct changes on sialography, and a history of intermittent parotid swelling. Of the two patients with Reiter's syndrome, one had definite keratoconjunctivitis sicca, but no other stigmata of Sjøgren's disease were found.

None of the 43 patients with osteo-arthritis was found to have SDA.

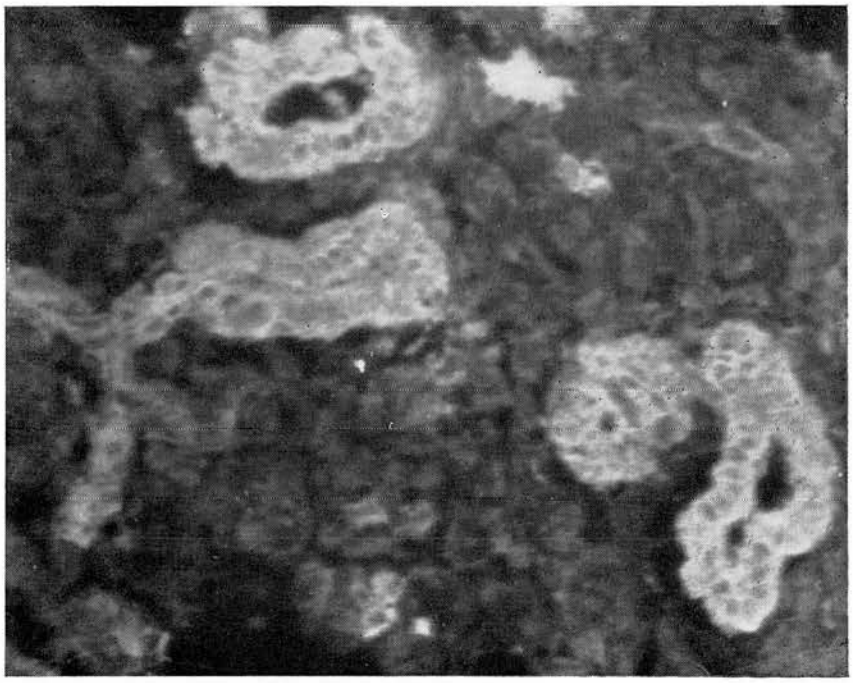
Sc and Sj-RA Groups (Tables II and III, overleaf)

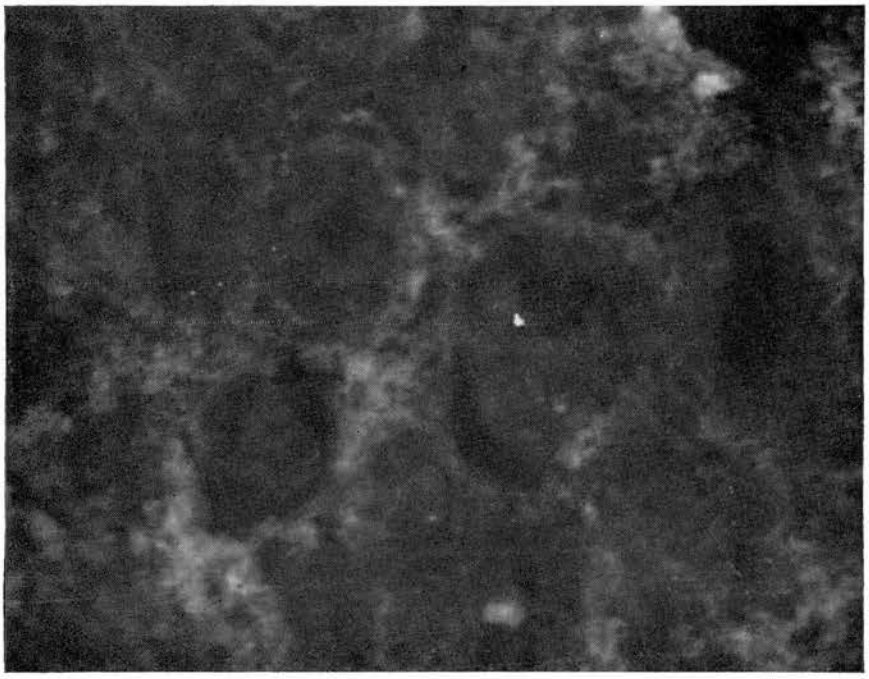
Patients with Sj-RA had SDA more often than did those with Sc ($\chi^2 = 5{\cdot}43$; $P<0{\cdot}02$). The two groups also differed in that the Sc patients were older ($t = 3{\cdot}89$; $P<0{\cdot}001$), and had more sialographic abnormalities ($\chi^2 = 12{\cdot}6$; $P<0{\cdot}001$) and a lower erythrocyte sedimentation rate ($t = 3{\cdot}47$; $P<0{\cdot}001$). In Sj-RA there was a negative correlation between SDA and ANF ($\chi^2 = 8{\cdot}24$; $P<0{\cdot}01$).

RA Group (Tables IV and V, Fig. 2, overleaf)

SDA was found significantly more frequently in older rheumatoid patients and in those with more severe rheumatoid disease as judged by functional grade, *x* ray stage, erythrocyte sedimentation rate, and the prevalence of rheumatoid factor. As shown in Fig. 2, the prevalence of rheumatoid factor for all titres except 1 in 32 as well as the highest titres were seen in SDA positive patients.

(*a*)





(*b*)

Fig. 1.—Frozen sections of human submandibular gland stained by indirect immunofluorescence
(*a*) With a serum containing salivary duct antibody: there is brilliant fluorescence of the duct epithelium, while the nuclei remain dark.
(*b*) With a normal serum: the ductal elements are just outlined

TABLE II
CLINICAL FINDINGS IN PATIENTS WITH SJØGREN'S DISEASE

Series			Sicca Syndrome (Sc)		Sjøgren's Syndrome and Rheumatoid Arthritis (Sj–RA)	
No. of Patients			13		17	
Salivary Duct Antibody			Present	Absent	Present	Absent
			2	11	11	6
Age (yrs)		Mean Range	64·0 62–66	64·6±8·3 53–78	56·8±11·3 33–63	55·8±16·6 29–55
Sex			2F	10F, 1M	8F 3M	5F, 1M
Keratoconjunctivitis Sicca	No. of Cases		2	11	11	5
	Duration (yrs)	Mean Range	4 2–6	7·9±6·7 1–21	8·7±8·4 <1–27	14·5±8·6 4–35
Xerostomia	No. of Cases		2	11	8	2
	Duration (yrs)	Mean Range	4 2–6	7·7±5·3 2–16	8·6±8·5 <1–25	13·3±9·9 5–31
Salivary Gland Enlargement			0	7	1	2
Abnormal Sialogram			2	10	3	2
Rheumatoid Arthritis	Duration (yrs)	Mean Range	—	—	8·5±8·7 5–27	15·2±11·1 6–35
	Functional Grade	I and II III and IV	— —	— —	9 (82%) 2 (18%)	3 (50%) 3 (50%)
	X ray Stage	I and II III and IV	— —	— —	6 (55%) 5 (45%)	2 (33%) 4 (66%)
	Subcutaneous Nodules		—	—	3	3

± Standard deviation

TABLE III
LABORATORY FINDINGS IN PATIENTS WITH SJØGREN'S DISEASE

Series		Sicca Syndrome (Sc)		Sjøgren's Syndrome and Rheumatoid Arthritis (Sj–RA)	
No. of Patients		13		17	
Salivary Duct Antibody		Present	Absent	Present	Absent
		2	11	11	6
Haemoglobin (g. per cent.)	Mean Range	13·2 13·1–13·2	13·5±1·6 10·6–15·7	11·9±2·1 8·7–15·4	11·7±1·8 9·7–14·1
Erythrocyte sedimentation rate (Westergren)	Mean Range	48 36–60	27·3±16·9 2–52	55·1±33·2 6–108	66·5±45·1 8–119
White Cell Count (cells per c. mm.)	Mean Range	4,550 3,900–5,200	5,267±1,440 3,200–7,650	6,633±1,000 5,200–7,600	6,655±2,040 2,900–10,700
Serum Globulin (g. per cent.)	Mean Range	4·0 3·7–4·2	3·9±0·42 2·4–4·3	3·75±0·42 3·1–4·5	3·8±0·54 3·3–4·7
Rheumatoid Factor Positive		1	6	8	6
Antinuclear Factor Positive		1	3	2	6
Non-specific Tissue Precipitin Positive		0	4	2	1
Thyroglobulin Antibody		0	3	3	1
Thyroid Microsomal Antibody		1	5	3	2
Gastric Parietal Cell Antibody		0	4	5	1

± Standard deviation

TABLE IV
RELATIONSHIPS BETWEEN SALIVARY DUCT ANTIBODY AND CLINICAL FINDINGS IN 129 PATIENTS WITH RHEUMATOID ARTHRITIS

Salivary Duct Antibody			Present	Absent	Significance
			34	95	
Age (yrs)		Mean Range	53·3±12·2 25–79	44·4±15·8 6–71	$t=2·85$ $P<0·001$
Sex			16F, 18M	57F, 38M	—
"Possible" Keratoconjunctivitis Sicca			15 (44%)	20 (21%)	$\chi^2=6·67$ $P<0·01$
Symptomatic Xerostomia			14 (41%)	9 (9%)	$\chi^2=17·18$ $P<0·001$
Abnormal Sialogram			4 (12%)	3 (3%)	—
Rheumatoid Arthritis	Duration (yrs)	Mean Range	6·5±6·4 1/12–50	6·6±8·3 9/12–50	—
	Functional Grade	I and II III and IV	20 (59%) 14 (41%)	79 (83%) 16 (17%)	$\chi^2=8·31$ $P<0·01$
	x ray shape	I and II III and IV	7 (21%) 27 (79%)	47 (49%) 48 (51%)	$\chi^2=8·58$ $P<0·01$
	Subcutaneous Nodules		7 (21%)	12 (13%)	

± Standard deviation

TABLE V
RELATIONSHIPS BETWEEN SALIVARY DUCT ANTIBODY AND LABORATORY FINDINGS IN 129 PATIENTS WITH RHEUMATOID ARTHRITIS

Salivary Duct Antibody		Present	Absent	Significance
		34	95	
Haemoglobin (g. per cent.)	Mean Range	12·9±1·6 8·1–16·2	12·8±1·9 8·6–17·0	—
Erythrocyte sedimentation rate (Westergren)	Mean Range	50±30·1 5–114	33±27·2 2–125	$t=2·94$ $P<0·001$
White Cell Count (cells per c. mm.)	Mean Range	7,800±2,640 3,100–15,300	7,570±2,300 2,900–17,700	—
Serum Globulin (g. per cent.)	Mean Range	3·9±1·2 2·4–4·6	3·37±0·6 1·9–4·9	—
Rheumatoid Factor Positive		29 (85·3%)	43 (45·2%)	$\chi^2=16·27$ $P<0·001$
Anti-nuclear Factor Positive		13 (38%)	20 (21%)	—
Non-specific Tissue Precipitins Positive		1 (3%)	4 (4%)	—
Thyroglobulin		3 (9%)	7 (7%)	—
Thyroid Microsomal Autoantibody		8 (23%)	17 (18%)	—
Gastric Parietal Cell Antibody		9 (26%)	11 (11%)	—

± Standard deviation

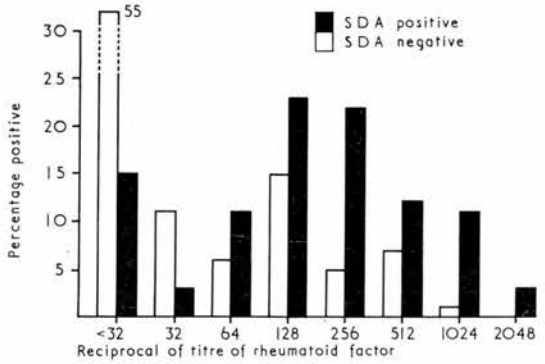


Fig. 2.—Histogram showing percentage of cases positive for rheumatoid factor in patients with rheumatoid arthritis with or without salivary duct antibody

A higher incidence of "possible" keratoconjunctivitis sicca and of symptomatic xerostomia was found in those with SDA. Table VI compares the frequency of "possible" keratoconjunctivitis sicca, symptomatic xerostomia, and abnormal sialograms in rheumatoid patients and in those with other rheumatic diseases—psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, gout, and osteo-arthritis. There is no significant difference. The sub-group of rheumatoid patients with SDA had symptomatic xerostomia more frequently than other patients ($\chi^2 = 6{\cdot}4$; $P < 0{\cdot}01$).

Comparison of Sj-RA and RA Groups

Among all the patients with rheumatoid arthritis, Sj-RA was found in those who were older ($t = 2{\cdot}1$; $P < 0{\cdot}05$), had had their arthritis for a longer period ($t = 1{\cdot}98$; $P < 0{\cdot}05$), and had more severe rheumatoid disease as judged by the erythrocyte sedimentation rate ($t = 3{\cdot}5$; $P < 0{\cdot}02$) and the presence of subcutaneous nodules ($\chi^2 = 4{\cdot}5$; $P < 0{\cdot}05$). The Sj-RA group also had a higher prevalence of SDA ($\chi^2 = 12{\cdot}23$; $P < 0{\cdot}001$) and of rheumatoid factor ($\chi^2 = 4{\cdot}37$; $P < 0{\cdot}05$).

Similarly, when Sj-RA patients were compared with SDA-positive RA patients, the former were shown to have had their arthritis longer ($t = 1{\cdot}73$; $P < 0{\cdot}05$) and to have a higher erythrocyte sedimentation rate ($t = 2{\cdot}4$; $P < 0{\cdot}02$). No age difference was, however, found.

Discussion

Bertram and Halberg (1964) first described the occurrence in Sjøgren's disease of an antibody against salivary duct epithelium. Since sera containing the antibody did not give immunofluorescent staining of salivary gland acini or of thyroid, they considered that the antibody might be specific for an antigen peculiar to salivary duct epithelium. Feltkamp (1967) has shown that the antibody could be absorbed from the serum with extracts of salivary gland, but extracts of a number of other tissues, including thyroid, liver, and kidney, failed to do so.

We have shown that the antibody reacts with the individual's own tissues (*i.e.* it is an autoantibody) and also causes immunofluorescent staining of small lacrimal ducts, but not of gastric, thyroid, or prostatic epithelium. The mitochondral antibody found in a high percentage of patients with primary biliary cirrhosis (Walker, Doniach, Roitt, and Sherlock, 1965; Goudie, Macsween, and Goldberg, 1966) gives an immunofluorescent staining pattern with salivary gland similar to that seen with SDA positive sera. Preliminary experiments, however, have shown that the SDA differs from the mitochondrial antibody in that only the latter can be absorbed from sera with rat liver mitochondria. The SDA thus shows some organ-specificity, but final confirmation must await further experimental investigation.

In our present studies we have found SDA in 15 per cent. of patients with Sc, but in 65 per cent. of

TABLE VI
"POSSIBLE" KERATOCONJUNCTIVITIS SICCA, SYMPTOMATIC XEROSTOMIA, AND ABNORMAL SIALOGRAMS IN RHEUMATOID ARTHRITIS AND IN OTHER ARTHRITIDES

Diagnosis	Rheumatoid Arthritis			Other Arthritides*
	Total	Salivary Duct Antibody		
		Present	Absent	
No. of Patients	129	34	95	67
"Possible" Kerato-Conjunctivitis Sicca	35 (27%)	15 (44%)	20 (21%)	20 (30%)
Symptomatic Xerostomia	23 (27%)	14 (41%)	9 (9%)	12 (18%)
Abnormal Sialogram	7 (5%)	4 (12%)	3 (3%)	4 (6%)

*Other arthritides = psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, gout, and osteo-arthritis.

patients with Sj-RA. In RA the incidence of the antibody was 26 per cent. In none of the large series of patients with osteo-arthritis was the antibody present. The antibody is thus not peculiar to Sjøgren's disease. It is most commonly found in Sj-RA, but is also present in one in four of patients with RA, and in one in seven of Sc patients. These observations suggest that the antibody is in some way related to the rheumatoid disease process, whether or not there be clinical evidence of salivary gland involvement. This is further emphasized in that, among RA patients, SDA was found significantly more frequently in older patients and those with more severe rheumatoid disease. Furthermore, Sj-RA also occurred in patients who were older, had had their arthritis longer, and had more severe rheumatoid disease. Circumstantial evidence of lacrimal and salivary gland involvement by the rheumatoid process was provided by the significantly higher incidence of "possible" keratoconjunctivitis sicca and of symptomatic xerostomia noted in the SDA positive RA patients as compared with the RA patients without the antibody. This might suggest that a subclinical form of Sjøgren's disease was present in the SDA positive RA patients.

Histological evidence of salivary gland involvement in rheumatoid arthritis was provided by Waterhouse and Doniach (1966), who found focal lymphocytic sialadenitis in all of twelve females and in four of five males with rheumatoid arthritis. They considered the salivary lesion regularly found in rheumatoid arthritis to be Sjøgren's disease in miniature. It is thus perhaps not entirely surprising that, in rheumatoid arthritis, without clinical evidence of salivary or lacrimal gland involvement, there should be a high incidence of salivary duct antibodies.

The finding of a significantly lower incidence of SDA in the Sc patients than in the Sj-RA patients is of considerable interest. The previous detailed studies of Bloch and others (1965), Beck and others (1965), and Bunim and others (1964)—summarized in Table VII—have shown differences between these two sub-groups of Sjøgren's disease. Furthermore, Talal, Leventhal, and Waldorf (1966) found that lymphocytic transformation in response to phytohaemagglutinin and streptolysin was less in Sj-RA than in Sc. However, with dinitrochlorobenzene skin sensitization, these workers found that differences between the two groups were not apparent. Reference to Table VII shows that, with the exception of anti-Gm factors, non-organ-specific autoantibodies have been found to be consistently more prevalent in the Sc patients. It is therefore surprising that in our present studies in patients with Sc, which clinically appears to be an organ-specific disease, the possibly organ-specific SDA should be significantly less common than in the Sj-RA patients. The number of Sc patients is small in our series, but our findings, taken in conjunction with the observations of other workers, clearly indicate the need for a more detailed comparison of Sc patients and patients with salivary and lacrimal gland involvement accompanied by a connective tissue disorder.

Summary

(1) An immunofluorescent autoantibody to salivary duct epithelium has been found in two of thirteen patients with sicca syndrome, in eleven of seventeen patients with Sjøgren's disease and rheumatoid arthritis, and in 34 of 129 patients with uncomplicated rheumatoid arthritis.

TABLE VII

COMPARISON OF SICCA SYNDROME (Sc) WITH SJØGREN'S DISEASE WITH RHEUMATOID ARTHRITIS (Sj-RA)
Bloch and others (1965) and Bunim and others (1964)

Diagnosis		Sc	Sj-RA	Reference
Serum Globulin (g./100 ml.)	Mean Range	4·4 2·8–6·8	3·6 2·0–5·7	Bloch and others (1965)
Antinuclear Factor		14/16 (88%)	14/25 (56%)	Bloch and others (1965)
Pattern of Nuclear Fluorescence Staining	Homogeneous	7/16	8/18	Bloch and others (1965)
	Speckled	5/16	2/18	
	Nucleolar	5/16	0/18	
Auto-immune Complement-Fixation Test		15/19	5/26	Bloch and others (1965)
Precipitating Autoantibodies		13/16	1/18	Bloch and others (1965)
Anti-Gm Factors		4/20	14/27	Bunim and others (1964)
Reticulum Cell Sarcoma		4/23	0/32	Bloch and others (1965)

Numerator = number of patients with positive tests
Denominator = number of patients tested

(2) In patients with rheumatoid arthritis, the antibody was found significantly more frequently in older patients and in those with more severe rheumatoid disease.

(3) The antibody appears to be a manifestation of the rheumatoid disease process, in which other workers have shown a high incidence of chronic focal sialadenitis.

We wish to acknowledge financial support from the Arthritis and Rheumatism Council, and from the Research Funds of the University of Glasgow.

One of us (M.K.J.) was in receipt of a CIBA clinical research fellowship.

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L'occurrence de l'anticorps contre l'épithélium du canal salivaire dans la maladie de Sjøgren, l'arthrite rhumatismale et dans d'autres arthritides; étude clinique et de laboratoire

RÉSUMÉ

(1) On trouva un anticorps immunofluorescent contre l'épithélium du canal salivaire chez deux sur treize malades atteints de syndrome *sicca*, chez onze sur dix-sept patients atteints de maladie de Sjøgren et d'arthrite rhumatismale et chez 34 sur 129 patients atteints d'arthrite rhumatismale sans complications.

(2) Pour l'arthrite rhumatismale, l'anticorps fut trouvé bien plus souvent chez des malades plus âgés et chez ceux dont la maladie rhumatismale fut plus sévère.

(3) L'anticorps semble être une manifestation du processus morbide rhumatismal dans lequel d'autres auteurs ont démontré une grande fréquence de la sialadénite focale chronique.

La ocurrencia del anticuerpo contra el epitelio del conducto salivario en la enfermedad de Sjøgren, la artritis reumatoide y en otros artritidicos; estudio clínico y de laboratorio

SUMARIO

(1) Un anticuerop inmunofluorescente contra el epitelio del conducto salivario fué encontrado en dos de trece enfermos con síndrome *sicca*, en once de diecisiete pacientes con enfermedad de Sjøgren y artritis reumatoide y en 34 de 129 pacientes con artritis reumatoide sin complicaciones.

(2) Respecto a la artritis reumatoide, el anticuerpo fué encontrado con frecuencia significativamente mayor en enfermos más viejos y en casos de enfermedad reumatoide más grave.

(3) El anticuerpo parece representar una manifestación del proceso morboso reumatoide en el cual otros autores han encontrado una frecuencia aumentada de la sialadenitis focal crónica.

Printed in England by DIEMER & REYNOLDS LTD., EASTCOTTS ROAD, BEDFORD

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Whaley, K. et al.: Acta Rheum. Scand., *14*, 298—308, 1968.

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LYMPHOCYTIC SIALADENITIS IN THE BUCCAL MUCOSA, IN SJÖGREN'S DISEASE, RHEUMATOID ARTHRITIS AND OTHER ARTHRITIDES

A Clinical and Laboratory Study

By

K. WHALEY, D. M. CHISHOLM, W. W. DOWNIE, W. C. DICK and J. WILLIAMSON

INTRODUCTION

Sjögren's syndrome, first described by the Swedish ophthalmologist Henrik Sjögren in 1933 (8), is now recognised as the triad of keratoconjunctivitis sicca (dry eyes), xerostomia with or without salivary gland enlargement and in one half to two thirds of patients rheumatoid arthritis or other connective tissue disease (1, 2). The keratoconjunctivitis sicca and xerostomia are due to reduced secretion by the lacrimal and salivary glands respectively, which are the seat of chronic inflammatory changes.

Approximately 10 to 15 per cent of patients with rheumatoid arthritis have keratoconjunctivitis sicca or some of its components (2) and approximately just over 25 per cent have mild xerostomia without evidence of diminished salivary secretions or abnormal sialograms (4).

An immunofluorescent autoantibody to salivary duct epithelium has been demonstrated in 15 per cent of patients with Sjögren's syndrome not associated with rheumatoid arthritis or other connective tissue disease, in 65 per cent of patients with definite Sjögren's syndrome and rheuma-

toid arthritis, and in 26 per cent of patients with uncomplicated rheumatoid arthritis. The autoantibody in the latter group of patients was related, however, to evidence of possible keratoconjunctivitis as judged by diminished tear secretion (7) and to symptomatic xerostomia. There was thus circumstantial evidence that the autoantibody to salivary duct epithelium reflected a subclinical form of Sjögren's disease (4). Waterhouse and Doniach (10) found focal lymphocytic sialadenitis in sixteen of seventeen patients with rheumatoid arthritis, and suggested that this lesion represented Sjögren's disease in miniature.

The present study was undertaken to determine the prevalence and nature of sialadenitis in the buccal salivary glands in Sjögren's syndrome with or without rheumatoid arthritis, and other arthritides.

MATERIALS AND METHODS

Patients

One hundred and eleven patients were studied. Table I shows the age and sex distribution and clinical diagnosis of these patients.

Sjögren's syndrome was diagnosed based on the criteria described by Bloch et al. (1): keratoconjunctivitis sicca, xerostomia with or without salivary gland enlargement, and rheumatoid arthritis or other connective tissue diseases. The diagnosis of Sjögren's syndrome required at least two of these three major components of the syndrome. The diagnosis of "sicca syndrome" was made in patients with keratoconjunctivitis sicca and xerostomia with or without salivary gland enlargement, but who had no evidence of rheumatoid arthritis or other connective tissue disease. The diagnosis of rheumatoid arthritis was based on the diagnostic criteria of the American Rheumatism Association (6). The majority of patients with rheumatoid arthritis, included in the study, whether associated with Sjögren's syndrome or not, had positive tests for rheumatoid factor and/or erosions on x-ray: all satisfied the criteria of "definite" or "classical" disease.

In addition to the age and sex of the patient the following clinical features were recorded: duration of arthritis, presence of subcutaneous nodules, functional grade and x-ray classification (9). Laboratory investigations included: full blood count, white cell count erythrocyte sedimentation rate (Westergren) and determination of serum globulin.

Each patient was examined for keratoconjunctivitis sicca by one of

TABLE I

The Incidence of a Focal Lymphocytic Sialadenitis in the Buccal Mucous Membrane in the Clinical Groups Studied.

	No. of patients studied	Sex		Age (yrs.)		With lymphocytic sialadenitis	
		Male	Female	Mean	Range	No.	Per cent.
Sicca syndrome	10	2	8	55.9	27—66	6	60.0
Rheumatoid arthritis and Sjögren's syndrome	20	3	17	60.1	48—77	13	65.0
Rheumatoid arthritis alone	40	10	30	54.1	20—73	11	27.5
Psoriatic arthritis	8	2	6	49.7	19—88	1	12.5
Ankylosing spondylitis	10	5	5	47.8	23—73	2	20.0
Reiter's syndrome	6	6	—	34.5	18—52	—	—
Still's disease	1	—	1	29.0	—	—	—
Systemic lupus erythematosus	1	—	1	21.0	—	—	—
Progressive systemic sclerosis	4	—	4	31.5	16—40	1	25.0
Gout	1	1	—	60.0	—	—	—
Osteoarthritis	10	3	8	64.9	53—74	1	10.0

us (JW) who was unaware of the clinical diagnosis, by the method described by Williamson et al. (12). A Schirmer I tear test was carried out in an atmosphere between 60 and 70°F. with a relative humidity greater than 40 (11) using standardised sterile paper strips supplied by Contactisol Inc., Lindenhurst, New York, U. S. A. Patients with wetting of the paper exceeding 15 mm. after 5 minutes were considered normal. Those with wetting less than 15 mm. had a rose bengal test and Schirmer II tear test. The rose bengal test was performed by instilling one per cent of the dye into the conjunctival sac, followed by irrigation with normal saline, and by examination with a Zeiss or Haag-Streit slit lamp for punctate and filamentary keratitis. Staining in the area previously in contact with the paper was ignored. The Schirmer II tear test consisted of a Schirmer I tear test while exposing the patient to a 10 per cent solution of ammonia held 6 inches from the nose for 5 minutes.

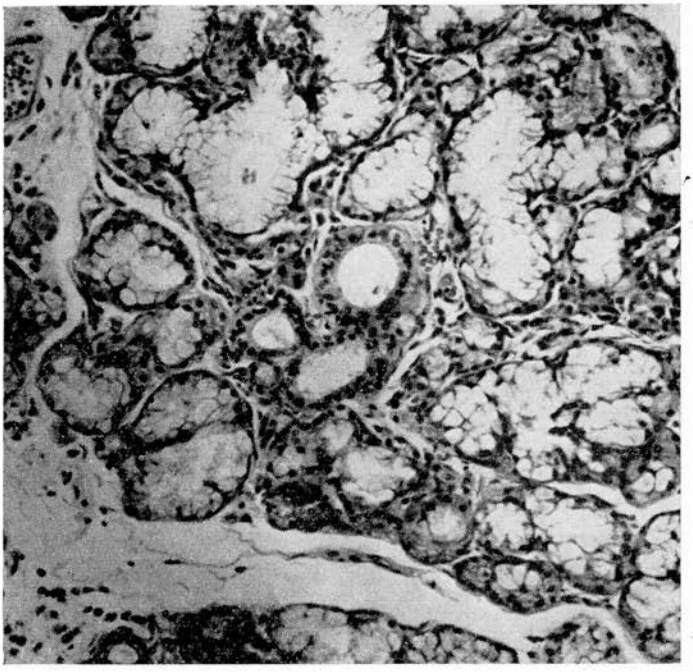

Fig. 1. Normal buccal mucosa. 190x.

A diagnosis of "definite" keratoconjunctivitis sicca was made only when both the Schirmer I and II tear tests showed wetting less than 15 mm., and punctate and/or filamentary keratitis was present on slit lamp examination. Patients with a "possible" diagnosis of keratoconjunctivitis sicca had a Schirmer I or II tear test less than 15 mm., but more than 5 mm. and the rose bengal dye test showed at the most only faint staining of the conjunctivae: patients with "possible" keratoconjunctivitis sicca did not have punctate and/or filamentary keratitis on slit lamp examination.

Each patient was carefully questioned regarding a history of xerostomia and associated dental symptoms of Sjögren's syndrome (1). Many patients stated that they had a dry mouth (symptomatic xerostomia) but had no diminished salivation and/or difficulty in mastication or deglutition requiring increased water intake. These patients' mouths appeared moist on examination and salivary flow studies using a modified Carlson-Criltenden cup were normal. In a previous study we had noted

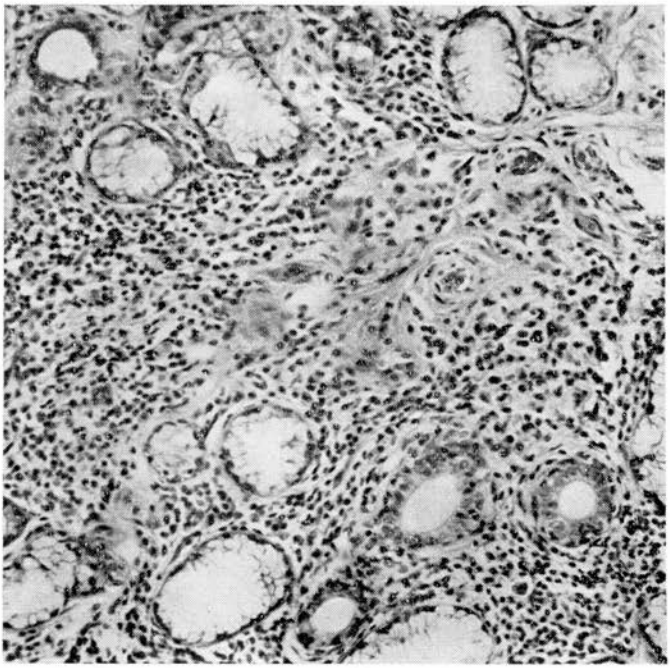

Fig. 2. Diffuse lymphocytic infiltrate. 190x.

that symptomatic xerostomia occurred in 27 per cent of patients with rheumatoid arthritis uncomplicated by overt Sjögren's syndrome as compared to 18 per cent of patients with other forms of arthritis such as psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, gout and osteoarthritis (4).

Sialography was performed using the hydrostatic technique of Park and Mason (5) in all 112 patients. The criteria of abnormality in the sialograms were based on those described by Bloch et al. (1).

RESULTS

The histological appearances of the buccal mucous membrane, were divided into 3 types, a) normal, fig. 1, b) a diffuse lymphocytic infiltration, fig. 2, and c) a focal lymphocytic sialadenitis, fig. 3.

Both diffuse and focal infiltrations could be further subdivided into two stages, based on the severity of the infiltrate (3). In this way five

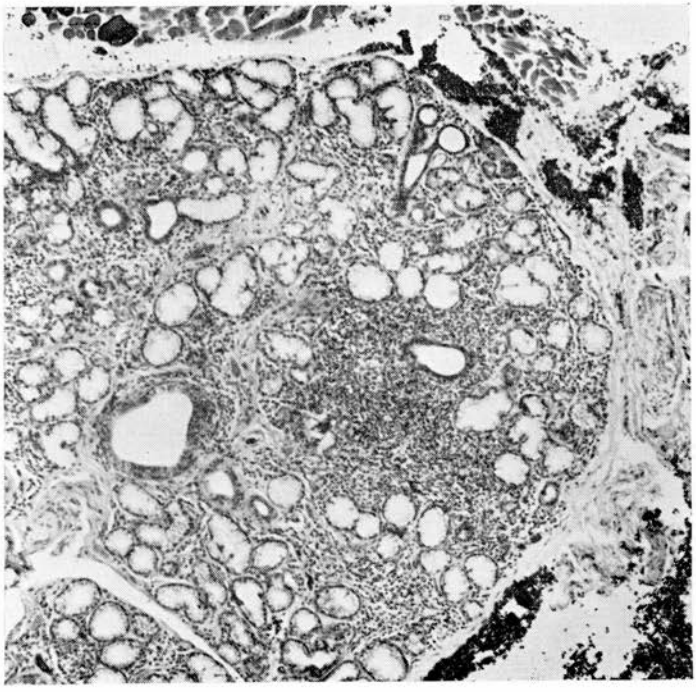

Fig. 3. Focal lymphocytic Infiltrate. 75x.

stages of lymphocytic infiltration in the buccal mucous membrane biopsy were identified.

Stage 0	— Normal biopsy appearance.
Stage I	— Fine diffuse lymphocytic infiltrate.
Stage II	— Marked diffuse lymphocytic infiltrate.
Stage III	— Focal infiltration of lymphocyte.
Stage IV	— Very heavy focal infiltration of lymphocytes.

Both stage III and IV, in addition to foci of lymphocytes, showed a diffuse infiltration of lymphocytes. Figure 4 shows the frequency with which the various stages of lymphocytic sialadenitis occur in the clinical groups included in the study. It can be seen that diffuse lymphocytic infiltrates are quite uncommon in all clinical groups. However, in post-mortem studies, stages I and II were quite common (3), and so these were not included as positive in this series.

Table I shows the incidence of positive biopsies in the groups included in the study.

TABLE II

The Relationship of Focal Lymphocytic Sialadenitis in the Buccal Mucosa and the Clinical Features of Patients with Rheumatoid Arthritis.

			Present	Absent
Focal lymphocytic sialadenitis			11	29
Age (yrs.)	Mean		51.5	55.6
	Range		20—73	39—73
Sex			10 F. 1 M.	20 F. 9 M.
Possible keratoconjunctivitis sicca			1 (9.1 %)	4 (13.8 %)
Symptomatic xerostomia			1 (9.1 %)	8 (29.4 %)
Abnormal sialogram			—	1 (3.4 %)
Rheumatoid arthritis	Duration	Mean	12.9	10.3
		Range	3/12—47	6/12—41
	Functional grade	I & II	2 (18.2 %)	5 (17.2 %)
		III & IV	9 (81.8 %)	24 (82.8 %)
	X-ray stage	I & II	2 (18.2 %)	5 (17.2 %)
		III & IV	9 (81.8 %)	24 (82.8 %)
	Articular index	Mean	32	25
		Range	1—66	4—64
	Nodules		2 (18.2 %)	8 (27.6 %)

In Sjögren's syndrome, with or without rheumatoid arthritis, the incidence of positive biopsies is virtually equal (65 % and 60 % approx.). Both of these are significantly higher than the incidence of 27.5 % in rheumatoid arthritis.

In the other arthritides, 2 patients with ankylosing spondylitis had positive biopsies, but neither had features suggestive of Sjögren's syndrome.

The one patient with osteoarthritis who had a positive biopsy, had a diminished Schirmer II test, but no evidence of rose bengal staining of the cornea. He was thus placed in the category of possible keratoconjunctivitis sicca, and this may explain the positive biopsy.

In psoriatic arthritis, 1 of the 8 patients included in the study had a

TABLE III

The Relationship of Focal Lymphocytic Sialadenitis in the Buccal Mucosa and the Laboratory Features of Patients with Rheumatoid Arthritis.

		Present	Absent	Significance
Focal lymphocytic sialadenitis		11	29	
Hemoglobin (G./100 ml.)	Mean	11.4±1.7	13.1±1.65	p 0.001
	Range	8.7—14.4	7.1—15.3	
White cell count	Mean	6,052±2,462	8,127±3,009	p 0.02
	Range	2,000—11,600	5,000—15,900	
Erythrocyte sedimentation rate (mm./hr. — Westergren)	Mean	78.9±19.3	41.6±26.1	p 0.001
	Range	36—107	5—106	
Serum globulin	Mean	3.9±0.65	3.5±0.68	—
	Range	3.2—5.5	2.6—5.2	

focal lymphocytic sialadenitis in the buccal mucosa. He also had possible keratoconjunctivitis sicca.

In the connective tissue disorders one of four patients with progressive systemic sclerosis had a stage IV infiltrate, however, this patient had no evidence of Sjögren's syndrome.

In table II, the clinical features of the patients with rheumatoid arthritis are shown. There is correlation between any of the clinical features and the presence of a focal lymphocytic sialadenitis in the buccal mucosa. This included the features of symptomatic xerostomia, and possible keratoconjunctivitis sicca, which may represent an early mild form of Sjögren's syndrome in rheumatoid arthritis (4).

The laboratory features of these patients are shown in table III. Patients with a focal lymphocytic infiltrate of the buccal mucosa had a significantly lower hemoglobin concentration, white cell count, and a significantly higher erythrocyte sedimentation rate, than those with a normal biopsy.

DISCUSSION

The incidence of a focal lymphocytic sialadenitis in the buccal mucosa, is very high in Sjögren's syndrome, with or without rheumatoid arthritis.

The incidence in rheumatoid arthritis is 27.5 %. However, there is no correlation with symptoms suggestive of early Sjögren's syndrome (possible keratoconjunctivitis sicca and symptomatic xerostomia). This may be due to examining the wrong salivary glands; the work of Waterhouse and Doniach (10) was performed on submandibular glands. In this respect, biopsy of lacrimal and parotid glands may give us a much better indication as to whether possible keratoconjunctivitis sicca and symptomatic xerostomia do in fact represent miniature Sjögren's syndrome, as is suggested by the work of MacSween et al. (4) on the salivary duct antibody. It is obviously of interest to further investigate the incidence of the salivary duct antibody in the patients studied and correlate its presence with the histological appearance of the buccal mucosa, and also to the other autoantibodies, which are known to occur frequently, and at a high titer in Sjögren's syndrome.

Acknowledgements

We wish to acknowledge the financial support of the Arthritis and Rheumatism Council, and the Research Funds of the University of Glasgow.

We are also indebted to Dr. W. Watson Buchanan for helpful advice during the preparation of this paper.

SUMMARY

The buccal mucous membrane biopsy has been examined histologically for focal lymphocytic sialadenitis, in patients with Sjögren's syndrome, Sjögren's syndrome with rheumatoid arthritis, rheumatoid arthritis alone, and other arthritides.

Focal lymphocytic sialadenitis was found in approximately 60 % of all patients with Sjögren's syndrome — with or without rheumatoid arthritis — and in 27.5 % of patients with rheumatoid arthritis. In rheumatoid arthritis, this did not correlate with symptomatic xerostomia, or possible keratoconjunctivitis sicca, both of which may be manifestations of a mild form of Sjögren's syndrome.

Fig. 4 for inclusion with the article Lymphocytic Sialadenitis in the Buccal Mucosa in Sjögren's Disease, Rheumatoid Arthritis and Other Arthritides, pp. 298—308, fasc. 4, 1968, Acta Rheumatologica Scandinavica.

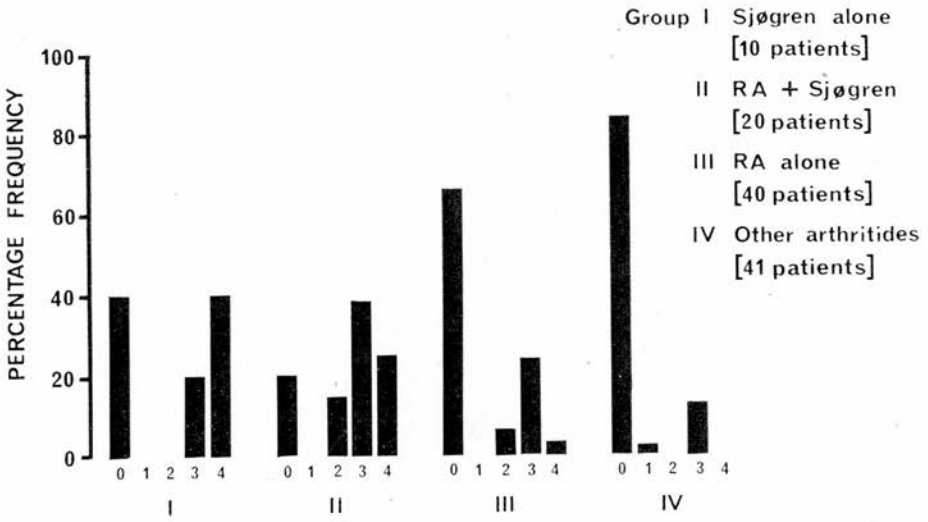

Fig. 4. A histogram showing the frequency of various grades of lymphocytic sialadenitis in Sjögren's syndrome, rheumatoid arthritis with Sjögren's syndrome, rheumatoid arthritis alone and other arthritides.

RÉSUMÉ

La biopsie de la muqueuse buccale a été explorée histologiquement pour l'inflammation lymphocytique focale des glandes salivaires chez des malades atteints du syndrome de Sjögren seul ou avec polyarthrite chronique évolutive, de polyarthrite chronique évolutive seule et d'autres arthrites.

L'inflammation lymphocytique focale des glandes salivaires a été constatée dans 60 % de tous les malades atteints du syndrome de Sjögren — avec ou sans polyarthrite chronique évolutive — et dans 27.5 % des cas de polyarthrite chronique évolutive. Dans ces derniers cas il n'y avait pas de corrélation avec la xérostomie symptomatique, ou la kératoconjunctivitis sicca, ces deux affections pouvant être des manifestations d'une forme bénigne du syndrôme de Sjögren.

ZUSAMMENFASSUNG

Die Biopsie der Mundschleimhaut wurde für fokale lymphocytische Sialadenitis histologisch an Patienten mit Sjögrens Syndrom mit oder ohne chronischer Gelekentzündung, mit Gelenkentzündung allein und mit anderen Gichtkrankheiten untersucht.

Die fokale lymphocytische Sialadenitis wurde bei ungefähr 60 % von sämtlichen Patienten mit Sjögrens Syndrom — mit oder ohne chronischer Gelenkentzündung — und bei 27.5 % von Patienten mit Gelenkentzündung festgestellt. Bei Fällen mit chronischer Gelenkentzündung gab es keine Korrelation mit der symptomatischen Xerostomie, oder einer etwaigen Keratoconjunctivitis sicca, welche beide als Erscheinungen einer milden Art von Sjögrens Syndrom betrachtet werden können.

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Salivary duct autoantibody in Sjogren's syndrome: correlation with focal sialadenitis in the labial mucosa

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Reprinted from
Clinical and Experimental Immunology
Vol. 4, No. 3, March 1969

BLACKWELL SCIENTIFIC PUBLICATIONS
OXFORD AND EDINBURGH

Clin. exp. Immunol. (1969) **4**, 273–282.

SALIVARY DUCT AUTOANTIBODY IN SJØGREN'S SYNDROME: CORRELATION WITH FOCAL SIALADENITIS IN THE LABIAL MUCOSA

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(*Received* 17 *September* 1968)

SUMMARY

No correlation between the occurrence of the salivary duct antibody and focal lymphocytic sialadenitis in the labial mucosa was found in ten patients with the sicca syndrome, twenty-seven patients with Sjøgren's syndrome and rheumatoid arthritis, and forty-seven patients with rheumatoid arthritis alone. No correlation between the variables was found in any of the groups examined. Post-mortem studies on the labial mucosal biopsy show that the results of biopsy are reproducible, and so the lack of correlation is not due to sampling error when the biopsy is taken. It is suggested that the salivary duct antibody is an epiphenomenon of rheumatoid arthritis rather than a manifestation of Sjøgren's syndrome *per se*.

INTRODUCTION

An autoantibody to the cytoplasm of salivary small duct epithelial cells has been demonstrated by indirect immunofluorescence in patients with Sjøgren's syndrome (Bertram & Halberg, 1964; Halberg *et al.*, 1965; MacSween *et al.*, 1967; Feltkamp & Van Rossum, 1968). This autoantibody is found in approximately 15% of patients with the sicca syndrome (keratoconjunctivitis sicca, xerostomia with or without salivary gland enlargement, but not rheumatoid arthritis or other connective tissue disease), 65% of patients with rheumatoid arthritis and Sjøgren's syndrome, and in 26% of patients with rheumatoid arthritis alone (MacSween *et al.*, 1967). Feltkamp & Van Rossum (1968), however, found the salivary duct autoantibody in the sera of approximately 50% of patients with the sicca syndrome and also in the same number of patients with Sjøgren's syndrome with rheumatoid arthritis. The reason why patients with rheumatoid arthritis who have Sjøgren's syndrome develop

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this autoantibody is unknown. Waterhouse & Doniach (1966) demonstrated focal lymphocytic sialadenitis in sixteen of seventeen patients with rheumatoid arthritis examined at post-mortem. The possibility, therefore, exists that the salivary duct autoantibody found in uncomplicated rheumatoid arthritis may reflect a subclinical form of Sjøgren's syndrome (MacSween *et al.*, 1967).

Since biopsy of the major salivary glands is not without risks, and since the labial mucosal glands are frequently involved in Sjøgren's syndrome (Bloch *et al.*, 1965; Chisholm & Mason, 1968), we thought it of interest to study the association between focal lymphocytic sialadenitis of the labial glands and salivary duct autoantibody in the serum of patients with Sjøgren's syndrome, rheumatoid arthritis or other arthritides and connective tissue diseases.

MATERIALS AND METHODS

One hundred and thirty patients were included in the study. The clinical diagnosis, age and sex distribution are shown in Table 1.

The diagnosis of Sjøgren's syndrome was based on the presence of any two of the three major components of the disease: keratoconjunctivitis sicca, xerostomia with or without salivary glands enlargement, and rheumatoid arthritis or other connective tissue disease (Bloch *et al.*, 1965). The diagnosis of 'sicca syndrome' was made when the patient had the first two of these components but did not have either rheumatoid arthritis or other connective tissue disease. Rheumatoid arthritis was diagnosed using the criteria of the American Rheumatism Association (Ropes *et al.*, 1958). All the patients with rheumatoid arthritis had 'definite' or 'classical' disease.

Ophthalmological examination

Each patient was examined by the method described by Williamson *et al.* (1967). One of us (J.W.) who was unaware of the clinical diagnosis, examined all the patients in the study. A Schirmer I tear test was carried out using standardized sterile paper strips (35 × 5 mm) developed by Halberg & Berens (Contactisol Inc., Lindenhurst, New York, U.S.A.), at a room temperature between 60° and 70°F, with a relative humidity greater than 40 (Williamson & Allison, 1967). After 5 min the length of wetting of the filter paper was measured, and the mean between the two eyes recorded. Those with wetting over 15 mm were regarded as normal, but those with wetting less than 15 mm had a Schirmer II test performed. This test is identical to the Schirmer I test, but lacrimation is stimulated using a 10% solution of ammonia, held 6 in. from the nose for 5 min. A 1% solution of Rose Bengal dye was instilled into the conjunctival sacs, immediately followed by irrigation with normal saline. The eye was then examined for evidence of a punctate or filamentary keratitis, using a Zeiss or Haag-Streit slit lamp. Staining in the area previously in contact with the filter paper was ignored.

'Definite' keratoconjunctivitis sicca was diagnosed by the presence of a diminished Schirmer I and II tear test, and a punctate or filamentary keratitis. Patients with diminished tear secretion, as shown by a subnormal Schirmer I and Schirmer II test but without evidence of a punctate or filamentary keratitis on slit lamp examination were classified as having 'possible' keratoconjunctivitis sicca.

Each patient was questioned closely for a history of xerostomia and any associated oral or pharyngeal symptoms of Sjøgren's syndrome (Bloch *et al.*, 1965). Salivary flow studies were performed on each patient using a modified Carlsson–Crittenden cup, with an outer chamber diameter of 20 mm and an inner chamber diameter of 10 mm. Only parotid saliva was collected under both resting conditions and following stimulation with fruit gums and lemon juice.

Many patients with rheumatoid arthritis complain of xerostomia, but without difficulty in swallowing or mastication, or increase in fluid intake. There is no evidence of xerostomia on oral examination, and salivary flow studies are normal. These patients are classified as having 'symptomatic' xerostomia (MacSween *et al.*, 1967).

Sialography, using the hydrostatic technique of Park & Mason (1966), was performed on all patients. The criteria of Bloch *et al.* (1965) were used as a basis for the diagnosis of abnormality.

Other clinical and laboratory data

Apart from the age, ophthalmological and oral examinations, the following laboratory investigations were also recorded: haemoglobin concentration, erythrocyte sedimentation rate (Westergren), white cell count and serum globulin level.

Serological methods

Salivary duct antibody (SDA). Human submandibular gland, obtained within 10 hr of death, was frozen to metal chucks with CO_2 snow, and 6-μ sections cut in a cryostat. The sera were applied undiluted to the unfixed section for 30 min at room temperature, then each section was washed with normal saline buffered with veronal (pH 7·2) for 10 min, followed by the application of fluorescein-conjugated goat anti-human globulin serum for 30 min. Finally the section was re-washed with buffered saline for 10 min, mounted in buffered glycerol, and examined under a Gillett and Sibert conference microscope, using blue light. The fluorescein-conjugated anti-human globulin serum was absorbed twice with dried rat liver to reduce non-specific fluorescent staining.

Antinuclear factor (ANF). Beck's indirect immunofluorescence method (1961) was employed, with rat liver as the substrate. The test sera were diluted 1:16 for initial testing, and positive sera were then titrated in quadrupling dilutions until an end point of nuclear staining was reached.

Anti-thyroglobulin. This was detected using the tanned red cell haemagglutination technique of Fulthorpe *et al.* (1961), using thyroglobulin coated formolized tanned sheep red cells (Burroughs Wellcome). Initially the sera were tested at a 1:16 dilution, and positive sera were titrated in quadrupling dilutions.

Thyroid 'microsomal' antibody. The indirect immunofluorescence technique of Holborow *et al.* (1959), using unfixed thyrotoxic thyroid tissue as substrate was used. The test sera were applied at a 1:4 dilution.

Gastric parietal cell antibodies were demonstrated by an indirect immunofluorescence technique (Adams *et al.*, 1964) using unfixed human gastric mucosa as substrate and undiluted test serum.

A highly reactive fluorescein-conjugated goat anti-human globulin prepared by Dr J. S. Beck was used for testing for the salivary duct antibody, but the other immunofluorescence

tests were performed using commercially prepared fluorescein-conjugated rabbit anti-human globulin (Burroughs Wellcome).

Rheumatoid factor was detected using the Hyland latex (R.A.) test technique (Hyland Laboratories, California) and also by the sheep cell agglutination test, a titre of 1:32 or greater being considered positive

Non-specific tissue precipitin tests were performed, using the method of Anderson *et al.* (1961) with human thyroid tissue as antigen. All sera were tested undiluted and at a 1:8 dilution.

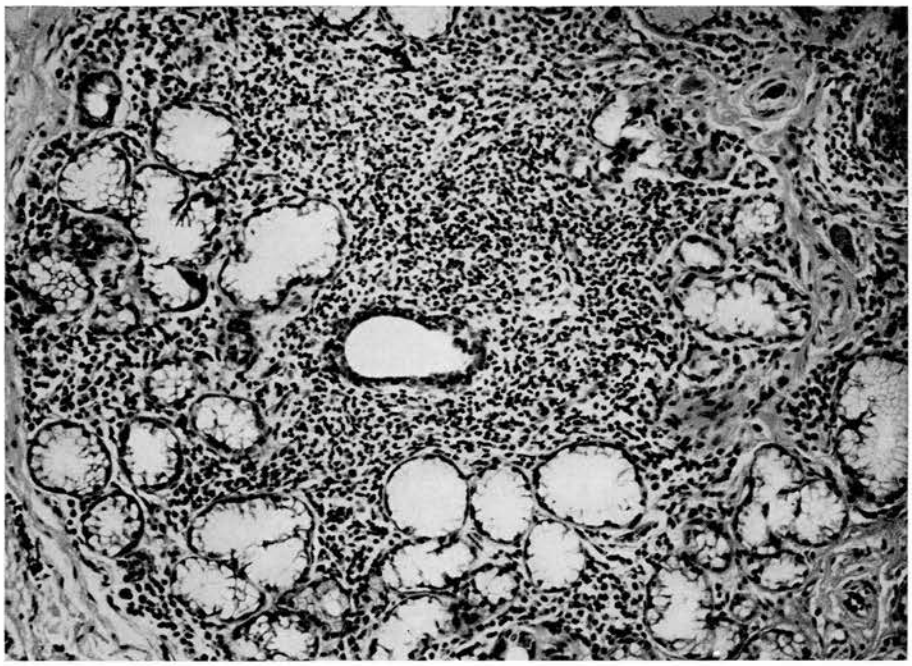

FIG. 1. Focal lymphocytic sialadenitis of labial mucosa. H & E, ×150.

Labial mucous membrane biopsy. The biopsy was taken from the lower lip under local anaesthesia (Chisholm & Mason, 1968). Focal lymphocytic sialadenitis was sought on 6-μ thick paraffin sections stained by haematoxylin and eosin. A focus of lymphocytes was defined as an aggregate of over fifty lymphocytes (Chisholm & Mason, 1968; Whaley *et al.*, 1969). A typical example of focal lymphocytic sialadenitis is shown in Fig. 1.

The autologous reaction of salivary duct autoantibody with the labial salivary glands was demonstrated in two patients using the indirect immunofluorescence technique (Fig. 2).

RESULTS

Table 1 shows the prevalence of salivary duct autoantibody and focal lymphocytic sialadenitis in the clinical groups studied.

Salivary duct autoantibody was present in 10% of patients with the sicca syndrome, in 70·4% of patients with Sjøgren's syndrome and rheumatoid arthritis, and 44·6% of patients

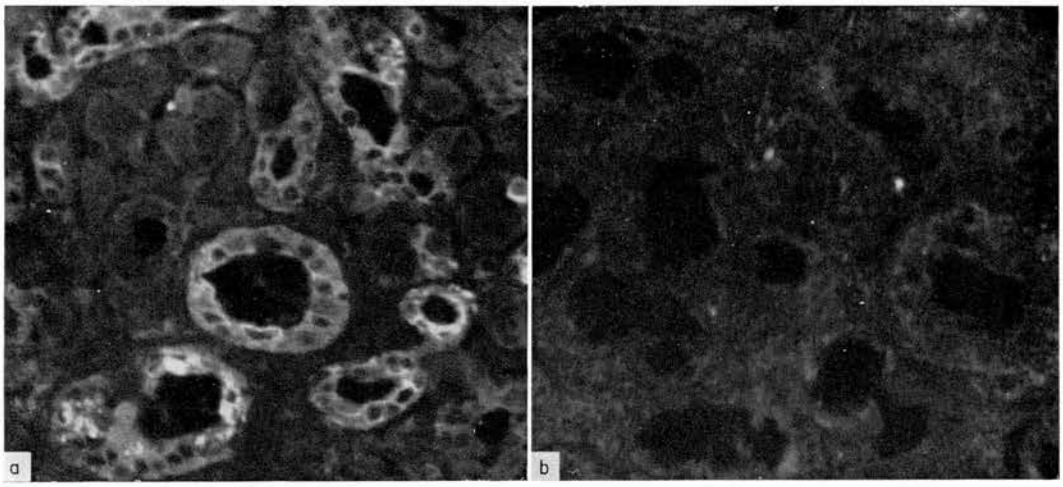

FIG. 2. (a) Autologous reaction of salivary duct antibody. Indirect immunofluorescent staining of labial salivary tissue from patient with Sjøgren's syndrome. Section treated with the patient's own serum which contained salivary duct antibody. (b) Control. Adjacent section to that shown in (a) but treated with normal serum instead of Sjøgren serum. No immunofluorescent staining of salivary ducts.

TABLE 1. Labial mucosal lymphocytic sialadenitis and salivary duct antibody in the clinical groups studied

Diagnosis	No. of patients studied	Sex		Age (years)		With lymphocytic sialadenitis		With salivary duct antibody	
		Male	Female	Mean	Range	No.	%	No.	%
Sicca syndrome	10	2	8	55·9	27–66	6	60·0	1	10·0
Rheumatoid arthritis and Sjøgren's syndrome	27	6	21	60·3	48–78	17	63·0	19	70·4
Rheumatoid arthritis alone	47	13	34	53·2	20–73	13	27·7	21	44·6
Psoriatic arthritis	11	4	7	49·3	19–88	2	18·2	—	—
Ankylosing spondylitis	10	5	5	47·8	23–73	2	20·0	1	10·0
Reiter's syndrome	6	6	—	34·5	18–52	—	—	—	—
Still's disease	1	—	1	29·0	—	—	—	—	—
Systemic lupus erythematosus	1	—	1	21·0	—	—	—	—	—
Progressive systemic sclerosis	4	—	4	32·0	16–40	1	20·0	—	—
Gout	2	1	1	61·0	60–62	—	—	—	—
Osteoarthritis	11	3	8	64·9	53–74	1	9·1	—	—

with rheumatoid arthritis uncomplicated by Sjøgren's syndrome. The prevalence of salivary duct autoantibody in patients with the sicca syndrome and Sjøgren's syndrome and rheumatoid arthritis is similar to that found in this laboratory by MacSween *et al.* (1967), but the prevalence of salivary duct autoantibody in patients with rheumatoid arthritis alone is higher than that reported by MacSween *et al.* (1967) (thirty-four of 129 patients, 26%).

Of the forty-six patients with other arthritides and connective tissue diseases only one had salivary duct autoantibody present. This was a 63-year-old woman with definite

TABLE 2. Laboratory and immunological features of patients with sicca syndrome

	Present	Absent	Significance
Focal lymphocytic sialadenitis	6	4	
Haemoglobin (g/100 ml)			
Mean ± SD	12·9	13·4	
Range	12·0–13·8	11·3–13·7	—
White cell count (per mm^3)			
Mean ± SD	5,850	6,150	
Range	3,300–7,800	5,000–9,700	—
Erythrocyte sedimentation rate (mm/1st hr—Westergren)			
Mean ± SD	24	15	
Range	14–38	3–32	
Serum globulin (g/100 ml)			
Mean ± SD	3·3	3·5	
Range	2·1–4·2	2·7–4·1	—
Salivary duct antibody	1 (15%)	0	—
Rheumatoid factor	5 (83%)	3 (75%)	—
Antinuclear factor	2 (33%)	1 (25%)	—
Non-tissue specific precipitating autoantibody	—	—	—
Thyroglobulin autoantibody	1 (13%)	2 (50%)	—
Thyroid microsomal autoantibody	1 (17%)	1 (25%)	—
Gastric parietal cell autoantibody	—	—	—

ankylosing spondylitis and a positive sheep cell agglutination test for rheumatoid factor at a titre of 1:256, but without clinical evidence of Sjøgren's syndrome or rheumatoid arthritis.

The prevalence of labial focal lymphocytic sialadenitis was the same in patients with sicca syndrome and Sjøgren's syndrome and rheumatoid arthritis (60 and 63%, respectively). The prevalence in rheumatoid arthritis alone was 27·7%.

Two patients with psoriatic arthropathy had focal lymphocytic sialadenitis, but neither had salivary duct autoantibody. One of these patients had 'possible' keratoconjunctivitis sicca.

Two patients with ankylosing spondylitis had focal lymphocytic sialadenitis, and one of

these, mentioned above, had salivary duct autoantibody present. Neither of these two patients had any evidence of Sjøgren's syndrome.

Of the four patients with progressive systemic sclerosis one had focal lymphocytic sialadenitis, but no evidence of Sjøgren's syndrome.

One patient with osteoarthritis had a positive buccal mucosal biopsy and had evidence of 'possible' keratoconjunctivitis sicca. No salivary duct autoantibody was detected in this patient's serum.

TABLE 3. Laboratory and immunological features of patients with Sjøgren's syndrome and rheumatoid arthritis

	Present	Absent	Significance
Focal lymphocytic sialadenitis	17	10	
Haemoglobin (g/100 ml)			
Mean ± SD	12·2	12·9	
Range	11·9–15·4	5·0–16·1	—
White cell count (per mm^3)			
Mean ± SD	6,946	6,297	
Range	3,400–14,600	1,260–10,500	—
Erythrocyte sedimentation rate (mm/1st hr—Westergren)			
Mean ± SD	42 ± 26·9	65 ± 42·9	
Range	12–121	8–125	—
Serum globulin (g/100 ml)			
Mean ± SD	3·6	3·8	
Range	1·8–4·5	2·9–5·2	—
Salivary duct antibody	12 (61%)	7 (70%)	—
Rheumatoid factor	16 (94%)	7 (70%)	—
Antinuclear factor	8 (47%)	3 (30%)	—
Non-tissue specific precipitating autoantibody	4 (23·5%)	—	—
Thyroglobulin autoantibody	3 (17·6%)	1 (10%)	—
Thyroid microsomal autoantibody	5 (29·4%)	3 (30%)	—
Gastric parietal cell autoantibody	4 (23·5%)	—	—

The relationship of the salivary duct autoantibody and other autoantibodies and laboratory data in patients with the sicca syndrome, Sjøgren's syndrome and rheumatoid arthritis, and rheumatoid arthritis alone is shown in Tables ~~2~~4. It can be seen that in none of these three groups did the salivary duct autoantibody correlate with focal lymphocytic sialadenitis. None of the other autoantibodies including rheumatoid and antinuclear factors, non-tissue specific precipitins, anti-thyroglobulin, anti-thyroid 'microsomes' and gastric parietal cell autoantibodies correlated with focal lymphocytic sialadenitis in any of the three clinical groups, with the exception of the antinuclear factor in patients with rheumatoid arthritis (Table ~~4~~) ($P<0·02$). None of the other laboratory features correlated with the

finding of focal lymphocytic sialadenitis in patients with Sjøgren's syndrome whether associated with rheumatoid arthritis or not. In patients with rheumatoid arthritis alone the haemoglobin ($P<0{\cdot}001$) and white cell count ($P<0{\cdot}02$) were significantly lower in patients with focal lymphocytic sialadenitis and the erythrocyte sedimentation rate was significantly higher ($P<0{\cdot}001$).

DISCUSSION

Sjøgren's syndrome is a chronic benign disorder which is characterized by chronic inflam-

TABLE 4. Laboratory and immunological features of patients with rheumatoid arthritis

	Present	Absent	Significance
Focal lymphocytic sialadenitis	13	34	
Haemoglobin (g/100 ml)			
Mean ± SD	11·3 ± 1·7	13·0 ± 1·65	
Range	8·7–14·4	7·1–15·3	$P<0{\cdot}001$
White cell count (per mm^3)			
Mean ± SD	6,108 ± 2,471	8,201 ± 3,023	$P<0{\cdot}02$
Range	2,000–11,600	5,000–15,900	
Erythrocyte sedimentation rate (mm/1st hr—Westergren)			
Mean ± SD	78 ± 19·3	41 ± 26·1	
Range	36–107	5–106	$P<0{\cdot}001$
Serum globulin (g/100 ml)			
Mean ± SD	4·0 ± 0·65	3·6 ± 0·68	
Range	3·2 –5·5	2·6 –5·2	—
Salivary duct antibody	5 (38·5%)	16 (47·1%)	—
Rheumatoid factor	13 (100%)	29 (85·3%)	—
Antinuclear factor	7 (53·8%)	6 (17·6%)	$P<0{\cdot}02$
Non-tissue specific precipitating autoantibody	3 (23·1%)	2 (5·9%)	—
Thyroglobulin autoantibody	5 (38·5%)	6 (17·6%)	—
Thyroid microsomal autoantibody	4 (30·8%)	7 (20·6%)	—
Gastric parietal cell autoantibody	2 (15·4%)	9 (26·5%)	

matory changes, not only in the lacrimal and major salivary glands, but also in the small mucus-secreting glands of the conjunctiva, mouth, nasal passages, pharynx, trachea and bronchi and also in the sweat glands (Bloch *et al.*, 1965). In this study ~~we have used~~ has been used biopsy of the labial mucous membrane to investigate the relationship of salivary duct autoantibody to focal lymphocytic sialadenitis of the oral mucosa in patients with Sjøgren's syndrome with or without rheumatoid arthritis, rheumatoid arthritis alone, and other arthritides and connective tissue disorders.

Salivary duct autoantibody was present in only one of ten patients with the sicca syndrome (Sjøgren's syndrome uncomplicated by rheumatoid arthritis or other connective tissue

disease), although six had focal lymphocytic sialadenitis on labial mucosal biopsy. In contrast, nineteen of the twenty-seven patients (70·4%) with rheumatoid arthritis and Sjøgren's syndrome had salivary duct autoantibody, although the prevalence of focal lymphocytic sialadenitis in this group was the same as in patients with the sicca syndrome. This suggests that salivary duct autoantibody may be a manifestation of Sjøgren's syndrome associated with rheumatoid arthritis rather than a reflection of Sjøgren's syndrome *per se*. This conclusion is supported by the finding of a very high prevalence of salivary duct autoantibody in patients with rheumatoid arthritis alone (twenty-one of forty-seven, 44·6%) and the comparatively low prevalence of focal lymphocytic sialadenitis in this group (thirteen of forty-seven, 27·7%). This high incidence of the salivary duct autoantibody probably represents a selection bias (MacSween *et al.*, 1967). Furthermore, there was a complete lack of correlation between salivary duct autoantibody and focal lymphocytic sialadenitis in patients with the sicca syndrome, Sjøgren's syndrome with rheumatoid arthritis, and rheumatoid arthritis alone. These findings are in agreement with those of Bertram (1967) who performed palatal biopsies on eight patients with Sjøgren's syndrome, six (75%) of whom had heavy lymphocytic and plasmacytic infiltrates and occasional myoepithelial cell islands. Of these six patients only two had salivary duct autoantibody present in their serum.

It may be argued that the lack of correlation between the salivary duct antibody and lymphocytic sialadenitis may have been due to a sampling error, the changes in the labial mucosa being patchy rather than generalized. However, in twelve post-mortem specimens, identical changes were found when biopsies were taken from both sides of the mouth. It is also possible that biopsy of the major salivary glands may have shown a correlation, but it is not feasible to carry out biopsy of these glands during life since the procedure carries definite risk of injury to the facial nerve, and of salivary fistulae, as well as leaving the patient with a scar. However, in post-mortem studies performed by Chisholm & Waterhouse (1968) a positive correlation between the findings in the submandibular and labial minor salivary glands could be made ($P < 0{\cdot}01$).

The salivary duct antibody reacts with the epithelial cytoplasm of the lacrimal and salivary glands and has been shown to have autoreactivity in one case (MacSween *et al.*, 1967). We have also shown autoreactivity in two cases. It has been shown by Feltkamp & van Rossum (1968) to be absorbed by extracts of salivary tissue but not by extracts of human pancreas, liver, thyroid, adrenal or muscle. The apparent organ-specificity of the antibody, therefore, corresponds to the occurrence of inflammatory lesions in lacrimal and salivary gland in Sjøgren's syndrome. Nevertheless, from our present findings, salivary duct antibody appears to be an epiphenomenon associated with the pathological changes of Sjøgren's syndrome complicating rheumatoid arthritis rather than with Sjøgren's syndrome occurring alone.

ACKNOWLEDGMENTS

We wish to acknowledge the guidance received from Dr W. W. Buchanan in the preparation of this paper. We would also acknowledge the financial support of the Arthritis and Rheumatism Council, and the Research Funds of the University of Glasgow. We are indebted to Dr J. Swanson Beck, University of Aberdeen, for supplying the fluorescein-conjugated goat anti-human globulin serum used in this study.

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